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INTERMITTENT BLOOD FLOW IN THE CAPILLARIES OF HUMAN SKIN[1]

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Submitted for publication July 1, 1937

INTRODUCTION

In 1926 Lewis (10) reported some observations "upon the regulation of blood flow through the capillaries of human skin" which led him to the conclusion that the tone of these vessels is relatively stable and unfluctuating. Most of his observations were made upon the skin of the back of the hand where he found that in a small area, constantly watched for an hour or more, the number and general appearance of the capillary loops remained essentially unchanged. Since no opening or closing of the loops could be detected, Lewis concluded that Krogh's (8) conception of the spontaneously intermittent activity of individual capillaries did not apply to the cutaneous capillaries of man. It is worthy of note, however, that what was actually recorded in Lewis' experiments was not the flow of blood in the capillaries, but, rather, the visibility of capillary loops. Many of the loops with which he was concerned were so dimly visible that they could be recognized only after prolonged and concentrated search. Lewis says that it was rarely possible to see the current of blood in these vessels. Thus his conclusions depend upon the assumption that capillary visibility is, in itself, a reliable indicator of capillary blood flow.

In order to determine more precisely whether the capillary circulation in the skin is truly intermittent, it would be advantageous to examine an area where the visibility of the minute vessels is suffi-

[1] Aided by a grant from The Helen Hartley Jenkins Fund. A report of some of these experiments was presented at a meeting of The Society for Clinical Investigation, May 6, 1935 (2).

[2] Jacques Loeb Fellow in Medicine.

ciently clear to allow one accurately to decide whether the contained corpuscles are in motion. The skin over the tibia is quite suitable for such a study. In this area, where the circulation is often very distinct, it has been found that intermittent capillary blood flow is a common phenomenon.

METHODS

Observations were made during the months of February, March, April and May upon 11 subjects, 8 males and 3 females. Their ages were 14, 16, 22, 23, 23, 28, 29, 30, 34, 36 and 45 years, respectively. Most of the experiments were performed upon three healthy males, aged 28, 30 and 34 years. The other eight subjects were convalescent patients in the wards of the hospital; all had normal arterial blood pressure, and, as far as could be determined, no cardiovascular abnormality.

During an experiment the subject lay flat in bed with a small pillow under the head. One leg, usually the right, was placed horizontally across a table, with the hip and knee extended and with the foot in slight external rotation. When the foot was supported in a comfortable position by means of small sand-bags most subjects experienced no difficulty in keeping the leg quiet for a period of several hours. The area of skin which was to come into view under the microscope was at a level about 5 cm. (2 to 6 cm.) below the surface of the sternum where it is joined by the second costal cartilage. A drop of oil or glycerin was placed upon the skin overlying the flat antero-medial surface of the tibia about 15 cm. above the internal malleolus. This area was then examined microscopically. Usually a binocular, double-objective microscope (magnification approximately $\times 85$), but occasionally a monocular microscope (magnification $\times 150$) was used. The field was illuminated by means of a 100 watt incandescent lamp, the condensed light of which passed through a blue-green filter. The lamp was about 1 ft. and the filter 6 ins. from the skin.

Most of the experiments were started two or three hours after the noon meal. The subjects had not been exposed to cold for at least several hours. Some of the convalescent patients, who had been lying in bed for many days, were carried from the ward to the laboratory in their beds in order to avoid any effects which might result from change in posture. Whenever it was necessary to shave the skin area which was to be examined, this was done always several hours and generally several days before the experiment.

Venous congestion, when required, was created by inflating a long blood pressure cuff, 21 cm. in width, which encircled the thigh just above the knee. The cuff was applied loosely at the beginning of an experiment so as not to disturb the leg during the course of the observations.

During an experiment, room temperature and skin temperature were recorded at intervals. The latter was determined by means of a thermal junction applied to the skin of the leg 3 or 4 cm. from the microscopic field.

The room temperature in all experiments was between 20.1° and 25.0.°[3] In the twenty-five experiments performed under resting conditions the variation in room temperature during a series of observations exceeded 1.0° in only four instances. The variation was usually only several tenths of a degree and it never exceeded 1.4°. All changes in room temperature were gradual, the extremes being separated by a period of more than an hour.

The extremes of skin temperature in the twenty-five experiments performed under resting conditions were 27.2° and 31.1°. Except in three experiments the temperature was constantly above 28° and it was usually about 30°. The lowest temperatures usually, but not always, occurred toward the end of an experiment. The variation in skin temperature during the course of a series of observations was 0.5° or less in eleven experiments. The maximum variation was 2.6° in a single experiment. The average change was about 1.0°. During periods of prolonged venous congestion the skin temperature tended to fall, but it remained above 28.4° except in one experiment when it fell to 26.3°. In the experiments in which efforts were made to raise or lower the skin temperature the extremes obtained were 35.0° and 21.5°.

The intermissions in capillary blood flow were recorded by one of two methods. When the first method was used, the observer called out his findings to a second person who sat nearby with watch in hand and made notes. The second method required a kymograph provided with three writing levers. One of the levers, the time-marker, indicated two-second intervals. The other two levers were controlled by the observer who, by pressing and releasing the appropriate keys, recorded the starting and stopping of flow in two capillaries. It is apparent that the reaction-time of the observer, and other personal factors, may play an important rôle in determining the accuracy of either of these methods. The percentage error due to such factors will diminish as the length of the intermissions increases. Both methods give reasonably reliable results when the intermissions are of longer duration than five seconds. The effort to make continuous observations simultaneously on more than two capillaries was so fatiguing and the results seemed so untrustworthy that such observations were attempted only when the visibility of the circulation was unusually clear.

With the exception of *diagram A* which is reproduced from a sketch in the protocol of an experiment, the drawings of the capillaries were made by an artist,[4] who worked with us almost daily. Our usual practice, before each series of observations, was to have a sketch made of the conspicuous vessels in the microscopic field. In the sketch we numbered the capillaries which we intended to watch for intermittent blood flow. These vessels were then reexamined in greater detail and any errors in the original sketch were corrected. In some experiments

[3] All temperatures here and elsewhere in this paper are recorded in degrees Centigrade.

[4] We are indebted to Miss Elizabeth Henrich of the Department of Art as Applied to Medicine for the careful attention which she gave to this work.

a glass coverslip, on which was engraved a $\frac{1}{10}$ millimeter scale, was placed over the microscopic field and the scale was included in the drawings.

RESULTS

Twenty-five experiments were carried out on different days under the natural resting conditions described above. The total number of capillaries on which blood-flow records were kept during the course of a single experiment varied from 2 to 13. Almost all of these capillaries were observed continuously for 10 minutes or longer. In several experiments two capillaries were under continuous observation for more than an hour.

In addition to the twenty-five experiments performed upon subjects at rest in warm surroundings further observations were made upon the same subjects under varying conditions of temperature, bodily posture and venous congestion.

General features of the capillary circulation

When the skin lying over the tibia is examined under resting conditions it is possible in most individuals to locate areas in which a clear view may be obtained of the circulation in the superficial venules and in a number of capillary loops.[5] Even in the most satisfactory subjects, however, it is not possible to see distinctly the vessels in every field examined, and in the majority of subjects it is necessary to examine many fields before finding one which is entirely suitable. Satisfactory fields are shown in figures 1 and 2. In figure 1 most of the branching, thread-like vessels are venules but there are several distinct capillary loops. In figure 2 two venules run diagonally across the field. Three capillary loops empty into the upper, and four into the lower venule. Two capillary loops not connected with either venule appear at the right of the drawing. In both figures arrows indicate the direction of the current of blood in the loops in which the flow could be clearly seen. Blood flow could also be seen in both of the long venules in figure 2 and in several of the venules in figure 1.

The junction of the venous limb of a capillary with the subpapillary venule is often clearly visible, but the arterial limb usually comes into view from the dim background and no arteriole can be seen. Occasionally one sees a short section of a vessel dividing to form two

[5] To familiarize oneself with the distribution of the capillaries in this area it is helpful to begin by examining the skin during venous congestion.

capillaries; such parent vessels will be called "arterioles," though they have not been properly identified by study of their histological structure. In rare instances (see figs. 6 and 7 and diagram A) relatively long arterioles may be seen.

The better visibility of the circulation in the capillaries of the leg, as compared with those of the dorsum of the hand, is dependent, in part at least, upon the following factors: (1) The relative transparency of the skin. (2) The obliquity of the capillaries' approach toward the skin surface,[6] which affords a view of longer stretches of arterial and venous limbs than can be seen in the dorsum of the hand. (3) The frequent occurrence of clear gaps in the column of circulating corpuscles, which renders the motion of the blood within the capillaries more readily detectable. The last of these factors is of great assistance, for when a capillary contains a column of crowded corpuscles, it is often extremely difficult to tell whether the blood is moving. The gaps, which are believed to be composed of transparent blood-plasma, are far more conspicuous in the minute vessels of the skin of the leg (see fig. 7) than they are in those of the dorsum of the hand or the nail-fold. An active capillary[7] in the leg usually displays a procession of relatively long cell-columns separated from each other by short clear gaps. As the capillary becomes inactive, there is a tendency for the gaps to lengthen.

The visibility of a capillary, under the conditions of these experiments, is wholly dependent upon its content of red blood corpuscles. The walls of the vessel cannot be seen, hence when the corpuscles disappear from view it is not possible to tell whether the capillary is collapsed and empty or whether it is full of stagnant or flowing plasma. Single corpuscles and groups of corpuscles have been seen floating through otherwise invisible loops, thus indicating that invisibility

[6] Schur (12) has previously commented upon and illustrated this peculiarity of the capillaries of the lower leg. He says that it may be accounted for by the broad flat character of the papillae. In other areas the course of the loops is directed toward the surface by small, steep papillae but here the capillaries travel in a horizontal plane, roughly parallel with the venule plexus.

[7] Here and elsewhere in this paper an "active" capillary is one through which corpuscles are streaming; an "inactive" capillary is one which contains motionless corpuscles.

does not necessarily signify collapse of the vessel. This might frequently lead to difficulties in interpretation were it not for the fact that a capillary, in which the motion of the corpuscles has, at any time, been clearly discernible, seldom disappears from view. When it does so, the period of invisibility, usually only momentary, appears to result from the passage through the loop of one of the long clear gaps mentioned above. Among all the capillaries of which we have records of circulation only a few have remained invisible for as much as 5 seconds in periods of observation of 10 minutes or longer. Only two have been continuously invisible for more than 20 seconds; one for 55 seconds, the other for 1 minute and 15 seconds.

Intermittent capillary blood-flow

When the circulation ceases in a capillary, one usually sees in the loop several clumps of stationary cells separated by clear gaps. Generally the tip of the loop contains an unbroken column of cells while the clear spaces tend to be more numerous in the two limbs. The portion of the loop which most frequently contains clear gaps is the arterial limb, especially that portion of it which lies farthest from the tip. Rarely does the flow stop in a capillary leaving it engorged with a continuous column of corpuscles. The cessation of flow may be quite abrupt or it may be preceded by a period of gradual slowing-down and thinning-out of the stream of corpuscles. Once the flow has stopped, the corpuscles and clear gaps almost always maintain their positions until flow is resumed, but at times there is a slow and hesitating drift toward the venous end of the loop. The resumption of flow may also be either gradual or abrupt. If gradual, a long clear space frequently enters the loop pushing ahead of it the corpuscles and gaps which have remained in the lumen during the period of inactivity. While this is in progress the capillary may become momentarily empty of cells but usually new corpuscles begin to enter the arterial limb before their predecessors have left the venous limb.

The drawings, diagrams and legends of figures 3 and 4 give information concerning the duration and frequency of intermissions in capillary blood flow as recorded in typical experiments. The following is an abbreviated protocol of another experiment:

Experiment 5–16. May 14. Subject: Housewife 45 years old. Being treated for mild anaemia. Haemoglobin 11.0 gms. per 100 c.c. Normal temperature, blood pressure and basal metabolic rate.

Subject lying flat in bed with right leg on microscope table. Surface of tibia 5.5 cm. below level of sternum at junction of second rib. Skin temperature taken just proximal to microscopic field.

"Visibility of capillaries excellent. Many loops showed intermittent flow. Capillaries No. 1 and No. 2 were unusually clear from arteriole to venule. Before the kymograph record was made, the blood flow was seen to stop several times,

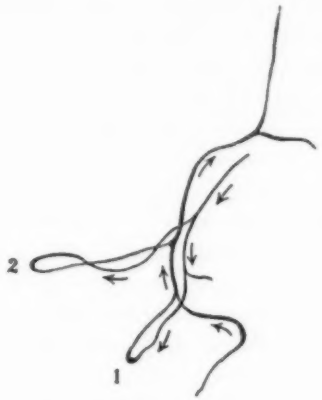

DIAGRAM A

first in one and then in the other of these loops. There was also a period of about a minute when the flow was inactive in both loops, stopping and starting simultaneously in the two. The drawing (diagram A) was made just before the kymograph was started."

TIME	ROOM TEMPERATURE	SKIN TEMPERATURE	REMARKS
p.m.			
3:05			Subject in position. B. P. 128/78
3:10	23.2		
3:35	23.0	29.0	
3:39:50			Kymograph started
4:08*			End of kymograph record
4:08:40	23.2	28.8	
4:27	23.3	28.6	(Experiment continued with kymograph records of other capillaries)

* Approximately.

The kymograph record was 27 minutes and 45 seconds in length. During this period the blood flow stopped in capillary No. 1 twenty-five times; the shortest intermission was less than 1 second, the longest 1 minute and 4 seconds. In capillary No. 2 the flow stopped seventeen times; the shortest intermission was less than 1 second and the longest 46 seconds. During the entire record capillary No. 1 was active 23 minutes and 31 seconds, and inactive 4 minutes and 14 seconds; capillary No. 2 was active 25 minutes and 39 seconds, inactive 2 minutes and 6 seconds. Except at one point, between the 5th and the 6th minutes, the intermissions in the two loops did not coincide, though occasionally they overlapped.

From the information contained in the foregoing protocol, and in figures 3 and 4 it may be seen that, with the subject at rest in warm surroundings and with the leg at heart level, interruptions in capillary circulation may be frequent and, at times, prolonged. The intermissions generally do not occur simultaneously in neighboring capillaries (fig. 4); in fact, even when two capillaries arise from a common arteriole, the flow may cease abruptly in one while proceeding rapidly in the other (experiment 5–16, above). Capillary behavior of the type described is so commonly observed that it may be considered a normal occurrence in the skin of the leg, under the conditions imposed.

One explanation of such behavior may be derived by assuming that individual capillaries are endowed with an occlusive mechanism which they may exert to prevent the passage of blood through their lumina. This hypothetical mechanism is so situated and so activated that it can interrupt the flow of blood through a single loop without detectably affecting the flow in the parent arteriole or sister capillaries. It may be possible, however, to explain the observations without presupposing an *active* occlusive mechanism. The circulation in a capillary is necessarily dependent upon the quantity of blood delivered by the parent arteriole and upon the pressure at which the delivery is effected. When the quantity or pressure of the arteriolar supply diminishes, it is conceivable that the flow will stop in small and elastic capillaries while it continues in their larger and less elastic neighbors. The intermissions in flow will, under these circumstances, be due to passive rather than active capillary resistance. The latter explanation appears to gain support from several experiments, exemplified by figures 6 and 7. In the diagrams of these figures it may be noted that the independent intermissions in flow all occurred in one of two capillary loops, while the other loop, arising from the same arteriole,

remained continuously active, or became momentarily inactive only at a time when flow stopped simultaneously in both vessels. If one should suppose—and the supposition is probably correct—that the quantity and pressure of the arteriolar blood supply varied during these experiments, then the behavior of capillaries no. 1 in figure 6 and no. 2 in figure 7 might be explained by the greater passive resistance to flow offered by these vessels. On the other hand, the results obtained in experiments such as 5–16, quoted above, are not easily accounted for by passive factors alone. Thus, the information obtained in experiments performed under natural conditions did not permit judgment concerning the relative importance of active and passive resistance in the production of intermissions in capillary circulation. For this reason additional experiments were carried out in which efforts were made to counteract the influence of passive resistance.

Effect of venous congestion on Capillary Circulation

Partial obstruction of the venous return from the leg should tend to overcome passive capillary resistance both by increasing the pressure within the minute vessels and by making available a larger amount of blood to fill the capillaries.[8] Accordingly, experiments were carried out in which the venous pressure in the leg was elevated by the use of a pneumatic cuff encircling the thigh (See methods). The cuff pressures used varied from 24 to 34 mm. Hg. Higher pressures were not employed because of the discomfort caused by their application for periods long enough to permit conclusive observations. Figures 5 and 6 show results typical of those obtained in all experiments. The results may be briefly summarized as follows: Venous congestion causes a slowing of the capillary circulation and some dilatation of all the minute vessels. Arterioles which have not been visible come prominently into view. The columns of corpuscles moving through the capillary loops become more crowded and take on a granular appearance. Nevertheless, the columns are still interrupted here and there by clear gaps between the clumps of cells. Intermissions in blood flow continue to occur and the character of the

[8] Landis (9) has shown, by direct measurement, that venous obstruction causes the capillary blood pressure to rise above the level of the increased venous pressure.

intermissions is the same as was noted before the venous pressure was raised. When a capillary becomes inactive, there is no damming-up of blood in the venous limb; in fact, an inactive loop usually contains less cells than an active one and, as shown in figure 6, may even become empty except for a few clumps of cells.

Similar observations were made in another series of experiments in which elevation of the trunk was used as a means of increasing the blood volume and blood pressure[9] in the leg. In these experiments the leg was at a level 25 to 30 cm. below that of the sternum where it is joined by the second costal cartilage. In some instances (see fig. 7) observations were made only with the leg below heart level. In other instances records of flow were kept on a selected group of capillaries with the leg at, as well as below, this level. The frequency, duration and general characteristics of the intermissions in capillary circulation appeared to be unaffected by these procedures.

The results obtained during venous congestion lead to the conclusion that any passive resistance which may be overcome by these means is not responsible for the intermissions in capillary circulation. Even in the presence of visible congestion of the minute vessels a mechanism is still available which may cause the flow to stop in a capillary and at the same time allow the inactive loop to become partially empty. Whether an increase in venous pressure modifies the frequency or duration of intermissions can not be deduced from the data obtained. The number and length of the intermissions which any capillary is capable of displaying are so unpredictable that significant modifications would not be detectable in a limited number of experiments. It is still possible, therefore, that venous congestion has some influence upon the occurrence of intermissions, but it does not prevent them, and it does not materially alter their characteristics.

The number of capillaries displaying intermittent blood-flow

Our inability to make observations on more than two capillaries simultaneously has prevented the accumulation of statistically valid information concerning the general prevalence of intermittent capillary

[9] The effect of this type of procedure upon the capillary pressure is described by Landis (9).

blood flow. The following numerical data embody a rough estimate of its occurrence.

While records were being made on two capillaries, intermittent flow was frequently noted in neighboring loops; on the other hand, whole fields of ten or more capillaries were occasionally seen in which not a single intermission was noted in a period of 15 minutes or longer. Out of 87 consecutive capillaries, in several subjects, observed for periods longer than 9 minutes each (average period of observation 14 minutes and 48 seconds), 42 displayed intermissions in which the individual pauses were longer than 10 seconds, while 34 showed continuous flow or intermissions of less than 2 seconds' duration. These figures would indicate that approximately 50 per cent of all capillaries observed for 10 to 15 minutes would show intermittent flow. This is probably too high an estimate, for in some of the experiments included in this series a frank search was being made for capillaries which would display the phenomenon. When successive capillaries were picked at random and watched continuously for 10 to 15 minutes each, the number showing intermittent flow was 3 out of 13 in one experiment, 6 out of 13 in another experiment, and 0 out of 8 in a third experiment; total 9 out of 34 (26%). In only one of the eleven subjects, a girl 14 yrs. of age, did we fail to find intermittent flow in at least some of the capillaries examined in each experiment. In this subject approximately the same area of skin was examined on three different days. On the first two occasions no capillaries were discovered which showed intermittent flow. On the third day, however, intermittent flow was observed in some of the capillaries in almost every field examined.

Effect of temperature

The irregular and unpredictable character of the circulation in single capillaries, together with our inability to make observations upon a large number of capillaries simultaneously, has defeated our efforts to determine the influence of temperature, and of other factors, upon the intermissions. The following indecisive information bears upon the effect of temperature.

Intermissions in flow have been noted at all room temperatures between 20.1° and 25.0°; they have been almost as frequent at the higher as at the lower temperatures. Likewise with skin temperatures varying from 27.2° to 31.1°, intermissions have been as frequent at the upper as at the lower of these levels. No attempt has been made to complete any experiments at room temperatures below 20.0°, for it was found that the subjects invariably became chilly and restless when exposed to such low temperatures for a period long enough to permit an adequate series of observations. Changes in skin temperature induced by direct application of heat or cold, unless extreme, gave equivocal results. Heating the

skin to the point of erythema appeared to diminish the intermissions, while cooling it rapidly by application of ice caused the flow to cease simultaneously in all the visible capillaries. Alterations in capillary circulation induced by such means may be totally unrelated to the spontaneously intermittent capillary activity observed under less extreme conditions of temperature.

Intermittent capillary-blood flow in other skin areas

Exploration of skin other than that of the leg, in search of intermittent blood flow, has not been extensive but it has been sufficient to indicate that the phenomenon is not a local peculiarity. Strictly comparable capillary behavior was, however, discovered in only one other area—the ventral surface of the forearm. The pertinent facts relating to three areas are summarized in the following paragraphs. The skin under observation was always at heart level, and the conditions were the same as those prescribed for the preceding experiments.

In two subjects records of flow were kept on a number of capillaries in the pale, relatively transparent, skin of the ventral surface of the forearm. The minute vessels in this area resemble in appearance those of the skin of the leg. Frequent and well sustained intermissions in flow were recorded in several capillaries in both subjects.

The skin of the dorsum of the hand appears to be less transparent than that of the leg or arm, and the small capillary loops rise perpendicularly toward the surface so that it is generally possible to see no more than their tips. The motion of the corpuscles may be indistinctly seen in a few loops, but in most of them it is not possible to tell whether the blood is stationary or flowing. In three experiments several capillaries were found in which the circulation was clearly enough visible to permit recording. In all, eight capillaries were watched for periods varying from 10 to 15 minutes each. Five of these vessels showed uninterrupted flow, two showed brief intermissions of less than 10 seconds, and one showed frequent and well sustained intermissions.

The capillaries of the nail-fold have, in the past, been more extensively studied than those of any other skin area. There are numerous conflicting reports (3, 5, 7, 10, 12, 14) concerning the occurrence of intermittent flow in these vessels. Many of the reports affirming its occurrence must be disregarded, as Lewis has pointed out, because of technical imperfections. Our own observations have failed to reveal intermissions similar to those seen in the leg. In the nail-fold the rate of capillary blood flow fluctuates: occasionally it slows down abruptly, or it may even stop momentarily, but rarely does it remain inactive for more than a few seconds. It may be recalled, however, that one of the conditions of these experiments is a warm environment, and that the vessels of the digits are highly respon-

sive to changes in the surrounding temperature. Continuous activity of the nail-fold capillaries may be demanded when the body requires dispersion of heat. The warm environment provided may therefore have prevented the occurrence of the phenomena which were sought in the experiments. Certainly, when the surroundings are cool, the blood flow in the nail-fold may become sluggish and it may stop intermittently in some capillaries. Whether these effects are wholly due to constriction of the precapillary vessels cannot be finally stated. It is possible that they are caused, in part at least, by a capillary mechanism which responds to the local metabolic requirements of the cool skin.

DISCUSSION

The observations here recorded indicate that the flow of corpuscles through individual capillaries in human skin may, under natural conditions, be controlled by a mechanism residing in the capillaries themselves. The control of the mechanism is almost certainly a local one, for it is difficult to conceive of a remote control which would affect adjoining or even neighboring capillaries so differently.

The existence of such an individual capillary mechanism, locally activated, has been doubted by Lewis (10) chiefly on the basis of experiments cited in the opening paragraph of this paper. In discussing the subject Lewis draws attention to the fact that "Krogh's generalization" upon the intermittent activity of individual capillaries was based upon experiments carried out under conditions which were abnormal for the tissues concerned: Krogh's own experiments upon frog tongue and muscle (8), Carrier's upon human skin (4), Richards' upon the frog's kidney (11), and Hagen's upon the rabbits' ear (6). Although not specifically mentioned by Lewis the numerous observations of intermittent capillary blood flow in human skin reported by Hinselmann and his associates (7) are open to the same form of criticism.

In our own experiments care was taken to avoid any influences which might be considered abnormal for skin. The conditions provided were those specified by Lewis as *natural* for the human subject. Nevertheless, one disturbing, and possibly *unnatural*, factor was recognized in almost all of these experiments: when the leg was kept motionless for the long period required for the observations there was a tendency for the skin temperature to fall. The temperature at which most of the observations were made was below what might be

considered "normal" for the region.[10] Three facts oppose the contention that this "abnormality" modified the results: (1) Intermissions were as frequent at the beginning, as at the end of an experiment. (2) The frequency and duration of the intermissions could not be correlated with the level of skin temperature. (3) Raising the skin temperature to 32–33° by artificial means (radiated heat of an electric lamp) did not appreciably alter the behavior of the capillaries. Aside from the enforced inactivity of the leg and the fall in its skin temperature no influences were recognized which might be considered *unnatural*.

Inasmuch as the capillary walls can not be seen in human skin, no information has been obtained concerning the nature of the mechanism which controls individual capillary blood flow. The distribution of the corpuscles during an intermission may be construed to indicate that the capillary wall has become constricted at one or several points. The slowing and thinning out of the stream of corpuscles which frequently precedes an intermission, and the almost invariable disappearance of the first portion of the arterial limb when the flow ceases, appear to indicate that the vessel has been occluded not far from the arteriolar-capillary junction. The events are similar to those which have been described in animal tissues where the capillaries can be more clearly seen (1, 11, 13). There the intermissions in flow have been ascribed to the contraction of Rouget cells or the expansion of endothelial cells (1).

SUMMARY

1. The flow of blood in the minute vessels of the skin of the human leg may be clearly seen under natural resting conditions.

2. The circulation in individual capillaries of this area is frequently intermittent.

3. Intermissions in flow may occur alternately in two capillaries arising from the same arteriole.

4. When the flow ceases in a capillary, even during venous congestion, the vessel may become almost completely empty of corpuscles.

5. These observations indicate that individual capillaries are en-

[10] The skin temperatures in Lewis' experiments (10) (24.6°–31.0°) were no higher than those observed in our subjects.

dowed with an occlusive mechanism which they may exert to prevent the passage of blood through their lumina.

6. Similar capillary behavior has also been observed in other skin areas.

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FIG. 1. MINUTE VESSELS OF SKIN OF LEG

Subject: male aged 34 years. Field 1 mm. square. All visible vessels were included in the drawing. In this and in subsequent figures arrows indicate direction of blood flow.

FIG. 2. MINUTE VESSELS OF SKIN OF LEG

Subject: female aged 14 years. Field 1 mm. square. Some of the visible vessels were omitted from the drawing.

Skin, rt. leg, over tibia Field = 1 mm. sq.

FIG. 1.

Skin, rt. leg, over tibia Field = 1 mm. sq.

FIG. 2.

FIG. 3. TWO DRAWINGS OF A GROUP OF CAPILLARIES WITH RECORDS OF BLOOD FLOW IN TWO LOOPS

(In this and in the following figures the time record indicates minutes. *Black* signifies continuation; *white*, means cessation of blood flow. A break in the record means that the observations were temporarily interrupted, usually to allow the artist to make observations.) Subject: male aged 30 years. Room temperature 22.1°–22.2°. Skin temperature 28.9°–28.8°. The drawings show five capillary loops near a point of crossing in the venule plexus. Flow was intermittent in all loops. Two loops, numbered 3 and 4, were selected for continuous observation, and the blood flow record, indicated in the diagram beneath the drawings, was made. Frequent intermissions appear in the records of both capillaries, the shortest several seconds, and the longest 15 minutes in duration. At the time of the lower drawing flow was active in both loops. The upper drawing was sketched during the break in the time record (14th to 15th minutes) when flow had stopped in capillary No. 4. Scattered groups of cells, separated by clear gaps appear in the inactive loop. These cells and gaps remained in exactly the same position for 15 minutes.

FIG. 4. TWO CAPILLARY LOOPS WITH RECORD OF BLOOD FLOW IN EACH

Subject: male aged 36 years. Room temperature 22.5°–22.0°. Skin temperature 29.3°–28.5°. The course of the arterial limbs of these capillaries was such as to indicate that they might both have arisen from the same arteriole. Both showed frequent, well-sustained intermissions in flow. The behavior of each loop seemed quite independent of the other, flow stopping abruptly in one while it remained vigorous in the other. (During the cross-hatched period the activity of capillary No. 2 was not being recorded.)

4.
3.
E. H. '35
CAP. #3
CAP. #4
0
MINUTES
5
10
15
20
25
30

FIG. 3

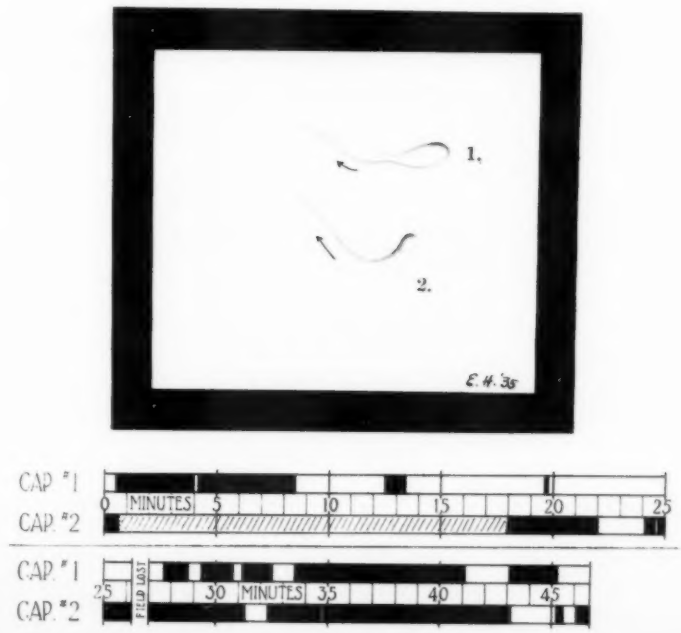

1.
2.
E. H. '35
CAP. #1
CAP. #2
0
MINUTES
5
10
15
20
25
CAP. #1
CAP. #2
25
FIELD LOST
30
MINUTES
35
40
45

FIG. 4.

FIG. 5. THE BEHAVIOR OF TWO CAPILLARIES BEFORE, DURING AND AFTER VENOUS CONGESTION

Subject: male aged 34 years. Room temperature 20.7°–21.6°. Skin temperature 28.6°–26.3° (latter during congestion). *A* shows capillaries during first gap in time record; flow active in both loops. *B*, *C*, *D* and *E* drawn during venous congestion, gap from 45th to 90th minute. *B* shows beaded appearance characteristic of congestion; circulation slow but active in both loops. In *C* no. 1 is active, no. 2 inactive. *D* shows a later intermission in no. 2, while no. 1 is still active. In *E* the venules and capillaries surrounding nos. 1 and 2 have been sketched in; at this time flow inactive in no. 1, active in no. 2. While drawings were being made, flow stopped in no. 1 from 52nd to 77th minute. (Congestion pressure in thigh cuff 29 mm. Hg for 58½ minutes. Conspicuous distention of veins beyond cuff.)

A
1.
2.
B
C
D
E
1.
2.
E. Heinrich '35

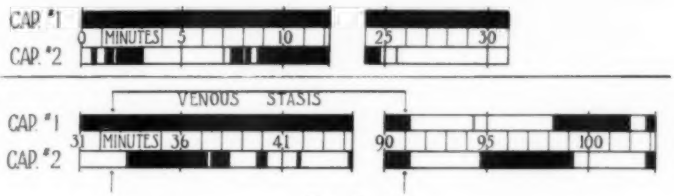

CAP. #1
CAP. #2
0
MINUTES
5
10
25
30
VENOUS STASIS
CAP. #1
CAP. #2
31
MINUTES
36
41
90
95
100

Fig. 5.

FIG. 6. TWO CAPILLARIES ARISING FROM THE SAME ARTERIOLE: EFFECT OF VENOUS CONGESTION

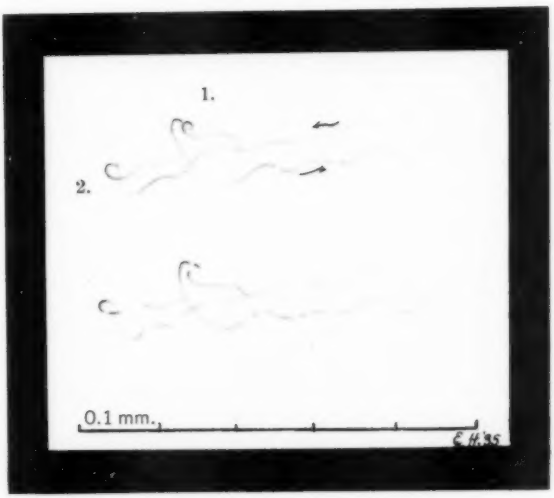
Subject: male aged 30 years. Room temperature 21.5°–21.8°. Skin temperature 30.6°–29.8°. Both drawings were made during the period of venous congestion; the one on the left while flow was active in both capillaries; the one on the right while no. 1 was inactive but no. 2 active. It may be noted that there was no damming-back of blood at the venous end of the inactive capillary; on the contrary, the vessel became empty of corpuscles except for a few stationary clumps in the tip of the loop. (The congestion pressure in the thigh cuff was 25 mm. Hg for 27½ minutes.)

FIG. 7. TWO CAPILLARIES ARISING FROM SAME ARTERIOLE: INCREASED HYDROSTATIC PRESSURE

Subject: male aged 30 years. Room temperature 22.8°–22.5°. Skin temture 30.6°–29.6°. The subject was lying with the leg horizontal, the trunk inclined slightly upward. Microscopic field 29 cm. below level of angle of Louis. Flow was active at the time of both drawings; the lower one pictures the vessels when numerous clear spaces were passing through them. The protocol notes that "as the flow stopped in no. 2 the column of cells seemed first to grow narrower, contained more clear spaces and the progress of the cells appeared slower. When the cells finally became stationary the ½ of the arterial limb lying closest to the arteriole was always invisible. . . . At one time when no. 2 was inactive, flow seemed unusually rapid in the arteriole, capillary no. 1 and the venule." The character of the intermissions was the same as had been noted with the leg at heart level.

1.
2.
E.H.'35
VENOUS STASIS
CAP. #1
CAP. #2
0 MINUTES 5 10 15 20 25 30
VENOUS STASIS
CAP. #1
CAP. #2
30 45 50 55 60 65

FIG. 6.


1.
2.
0.1 mm.
E.H.'35

CAP. #1
CAP. #2
0 MINUTES 5 10 15 20

FIG. 7.

STUDIES IN HAEMOLYTIC ANAEMIA

I. Haemolysis, Compensatory Regeneration and Erythroblastosis

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The first step toward an understanding of the mechanism by which haemolytic anaemia develops is a study of the equilibrium between blood formation and destruction, how it is maintained and how, when it is disturbed, compensation is attained so that life may go on. Before we can advance, however, it is essential to state the equilibrium in quantitative terms, *i. e.* to measure the rate of blood formation and destruction and to balance one magnitude against another. In papers published previously, I have attempted to justify the use of the urobilin excretion in the stools as a means of measuring blood destruction (1, 2, 3). In these papers it was shown that the urobilin excretion, when expressed in relation to the total circulating haemoglobin, was relatively constant in normal individuals (1), and could be used as an index of blood destruction[1] to balance the reticulocyte percentage used as an index of blood formation (2, 3). The purpose of the present paper is to apply this method to cases of haemolytic anaemia.

The difficulties are twofold: In the first place there is the uncertainty inherent in the methods of measurement by which we must infer relative rates of formation or destruction from figures for reticulocytes and urobilin excretion. (2) The equilibrium as we measure it may be disturbed by certain processes which influence the validity of the methods used for measurement. When haemoglobin is rising,

[1] The use of this index of urobilin excretion in various forms is becoming widespread in European countries, especially Germany and Italy; but as far as I am aware the attempt to balance formation against destruction quantitatively has not been attempted.

it is not impossible that some portion of the products of pigment catabolism may be retained to some extent to be used in the new formation of haemoglobin.[2] On the other hand, the presence of erythroblasts brings into question our right to use the reticulocyte percentage as a measure of the rate of formation when there is manifest difficulty on the part of the haemopoietic tissue in bringing cells to complete maturity. The first part of this work will deal with these two questions, especially that of the disturbance introduced by the presence of erythroblastosis.

MATERIAL AND METHODS

The material on which this report is based comprises a number of cases of sickle cell anaemia, congenital haemolytic jaundice, acute haemolytic anaemia, and a few other cases of rather obscure origin. Because of the necessity to save space it has been found impossible to include all the figures from the individual cases. In the part devoted to the case histories are a few tables of the nature of protocols. These are sufficient to illustrate the most important conclusions, as well as to give an idea how the study was conducted. The data from the omitted tables are largely confirmatory. Table 1 summarizes the data, and some of the special points are illustrated in the charts. Likewise in the clinical descriptions much that is not relevant to this study has been omitted. The clinical aspects of the conditions represented by the cases being studied are well known and little would be gained by recapitulation, so that in the description of the cases the chief emphasis has been placed only on the details that have a bearing on the present study.

The methods used are standard and need no description. The method for determination of urobilin has been recently described (1). The reticulocyte counts were made in a wet preparation, and the percentage approximately estimated, since it was felt that such an estimation gave the essential information. Urobilin estimations were made on stool specimens representing two- and three-day excretions. Fluctuation in two-day specimens in older children is considerable and it is necessary to average two or more of these consecutive short

[2] The possibility of pigment retention was discussed in a previous paper (1) in relation to very low values for urobilin excretion following infection.

periods to obtain smooth curves. This has been done for the figures reported in the tables, thereby also saving space. It has been found that calculation of urobilin excretion on the basis of the estimated grams of circulating haemoglobin gives a better understanding of the relative rate of haemolysis than the use of the absolute figures for urobilin excretion. A discussion of this may be found in a previous paper (1).

Some of the periods of remission were initiated by a method of treatment that will be described in a subsequent paper, and some of the apparent exacerbations were merely returns to the previous state after the induced remission had run its course. For this reason, the fluctuations are greater than they would be under ordinary circumstances.

UROBILIN EXCRETION AND BLOOD DESTRUCTION

It has never yet been proved that urobilin excretion measures blood destruction. The best that has been done is to show that in individual cases it acts as if it were approximately proportional to the rate of blood destruction. An objection to this has been advanced by Castle and Minot (4), who point out that, if this were so, then in pernicious anaemia in relapse the rate of blood destruction would have to be considered as increased ten to fifteen times above the normal. On the other hand, pathological studies indicate that blood formation is so interfered with that it would be impossible for anything even approaching equilibrium to be established. But unless equilibrium were established, the red cells would continue to fall and life would not go on. They therefore deny that urobilin excretion is a measure of blood destruction in this condition. This objection can not be met at the present time because of an inadequate study of pernicious anaemia from this point of view. It may be, however, that haemoglobin or its forerunners are being built up more rapidly than they can be used and the excess may be destroyed, giving rise to the excess urobilin. Another possibility is that destruction is taking place at a level of red cell formation below the point at which the cells enter the circulation.

The work reported here is based on the proposition that urobilin excretion is a measure of blood destruction. This proposition is just

as much incapable of proof through the observations reported here as it has always been, but the relations of reticulocyte percentage and urobilin excretion to the fluctuation in red cells and haemoglobin are adequately explained on this basis, as will be seen later. The extension of this proposition to other forms of anaemia is not entirely justified at the present time, though from past and present experience I should be inclined to believe that any account of anaemia that ignores an increased total urobilin excretion as an indication of increased breakdown of haemoglobin falls short of a complete explanation of the facts.

RETICULOCYTES AND ERYTHROBLASTOSIS

The second problem concerns the right to use the reticulocyte percentage as a measure of the rate of blood formation, and the part played by erythroblastosis. In the former work to which reference has been made, the conditions were such that an equilibrium was approximated with some frequency—changes were not very marked and not very rapid. In the cases of haemolytic anaemia studied here urobilin excretion and reticulocyte percentage were continually undergoing variations, so that it became difficult to compare values that belonged together. On studying the charts more closely it was found that there was usually a very definite lag in the reticulocyte response to increased blood destruction, so that a peak in urobilin excretion was usually followed by a peak in reticulocyte percentage, and a trough by an equivalent fall in reticulocytes (see Chart 4). By using this relationship, it has been possible to chart the reticulocytes against corresponding urobilin excretion, and to show that in the absence of erythroblasts there exists an approximate ratio between urobilin excretion and reticulocyte percentage and that this ratio approximates that found previously to represent the state of equilibrium (2), namely:—Urobilin (mg. per gm. hgb.): Reticulocyte (per cent) = .4 − .7 (Chart 1).

As may be seen from Chart 1 this relationship is completely shattered by the presence of erythroblasts. In my own experience, as indicated by the chart, an excessive reticulocyte count has invariably been coupled with the presence of erythroblasts. In the literature while, generally, high reticulocyte counts have been associated with

TABLE 1

This table is an attempt to bring together the urobilin excretion and reticulocyte percentage that appear to belong together, taking into account the lag in reticulocyte response. Also, during periods when there are no rapid fluctuations in urobilin excretion, the general level of urobilin excretion, reticulocyte percentage, etc., has been averaged.

Explanatory notes. (1) In the second column are contained (a) the average urobilin excretion over periods during which a fair degree of equilibrium existed; (b) the maximum urobilin excretion during an exacerbation and the minimum during a remission. The date on which this maximum or minimum was observed is contained in Column 1.

(2) The reticulocyte percentage is that that appears to correspond to the urobilin excretion of Column 2 taking into account the lag in reticulocyte response.

(3) Likewise the values of R.B.C. and Hgb. are those that have resulted from the increase or decrease of urobilin excretion during the period of exacerbation or remission represented by the urobilin excretion of Column 2.

(4) The symbols in this column are intended to give some idea of the magnitude of rise or fall in red cells or hemoglobin during the remission or exacerbation represented by the urobilin excretion of Column 2.

CASE NO.	YEAR	DATE (UROBILIN EXCRETION)	UROBILIN EXCRETION (1)	RETICULOCYTES, PER CENT (2)	LAG IN RETICULOCYTE RESPONSE	R.B.C. (3)	HGB. (3)	ERYTHROBLASTOSIS	RISE OR FALL (4) IN R.B.C.	
1	1932	11/ 7–12/20	8.2	11	—	1.8	5.9	0	—	
	1933	10/19–12/23	8.9	16	—	1.9	7.3	0	—	Average for period
		11/30	6.1	12	9	2.4	7.7	0	+	Remission
2	1933	9/29–10/11	11.5	15	—	2.6	7.4	0	—	Average for period
		10/20	4.5	14±	5?	3.2	8.9	0	+	
	1934	1/15	15	25	5±	1.7	5.6	0	++	Crisis
		1/22	7.5	10	5	2.7	7.9	0	++	Brief remission
		1/28	18	25	9	1.4	4.4	0	++	Crisis
		2/14	17	30	8	2.5	7.7	0	++	
3	1932–3	12/24– 5/5	15	35	—	2.0	5.0	++	—	Average early period
		5/ 5– 7/4	7	25	—	2.7	7.0	+	—	Average later period
		12/26	2.9	25	7	3.1	6.9	+	+	Remission following transf.
		1/14	13	60	3	1.7	4.5	++	++	Crisis
		2/14	11	25	0	2.3	5.3	±	+	Remission with disappearance of erythroblastosis
		2/21	17	60	0	1.6	4.2	++	+	Crisis
		2/28	12	25	10	2.3	5.1	±	+	
		4/8	29	60	0	1.7	4.1	+++	+	
		5/3	25	50	0	1.8	5.3	++	0	See chart 6
		5/20	8.5	30	+	2.6	6.6	++	+	
		6/26	5.3	20	0	2.7	6.8	+	0	

TABLE 1—*Continued*

CASE NO.	YEAR	DATE (UROBILIN EXCRETION)	UROBILIN EXCRETION (1)	RETICULOCYTES, PER CENT (2)	LAG IN RETICULOCYTE RESPONSE	R.B.C. (3)	HGB. (3)	ERYTHROBLASTOSIS	RISE OR FALL (4) IN R.B.C.	
3	1935	2/ 5– 3/25	7.5	20	—	2.7	7.5	±	—	Average for period
		3/23	6.6	12	5	2.7	7.7	±	+	See chart 4
		3/28	18	35	6	1.8	5.4	+	++	
		4/11	6.5	15	6±	2.5	7.8	±	++	
4		3/22– 7/30	14	35±	—	1.8	4.5	++	—	Average for period
		3/28	17	40	6	1.4	3.5	++	—	
		4/2	6.5	25	7	2.0	5.7	++	++	
		4/7	13	37	7	1.5	4.5	++	++	
		4/13	10	18	6	3.1	7.6	+	++	
		4/17	14	34	9	1.6	4.6	+++	++	
		5/22	18	50	0	1.8	3.9	++	—	Period with high urobilin excretion and erythroblastosis
		6/23	7.2	25	0	2.5	5.5	+	+	See chart 5
		7/25	14	50	0	1.8	4.1	+	0	
5	1935	3/28	7.4	55	0	2.1	8.7	+	+	
		4/5	4.0	25	5	2.5	9.7	+	+	
		10/ 2–12/18	12.5	45	—	1.3	4.5	++	—	Average for period
		11/1	10	14	18–	1.8	5.1	+	+	
		12/7	21	88	0	1.0	3.2	++	0	
	1936	2/8	4.2	8	0	3.0	9.2	0	—	After splenectomy
		2/29	8.8	52	0	1.3	5.3	+++	++	
6	1930	12/10	14.5	40	0	—	5.2	++	+	
	1931	1/20	2.2	42	10	—	5.0	++	++	
		2/2	6.4	12	9	—	7.1	0	++	
		2/26	13	40	11	—	6.0	+++	+	
		4/13	4.3	7	0	—	7.9	0	+	
		4/24	11	75	0	—	6.1	++++	++	
		6/8	3.1	10	0	—	7.4	0	—	Gradual recovery
7	1931	5/18– 6/16	3.5	10	—	—	8.0	0	—	Average for period
		5/9	14	40	—	—	3.5	++	—	On admission in a crisis
		5/25	3.1	7	—	—	7.6	0	—	
		6/3	5.6	12	0	—	8.0	0	0	
		6/16	2.0	6	0	—	7.1	0	—	

TABLE 1—*Concluded*

CASE NO.	YEAR	DATE (UROBILIN EXCRETION)	UROBILIN EXCRETION (1)	RETICULOCYTES, PER CENT (2)	LAG IN RETICULOCYTE RESPONSE	R.B.C. (3)	HGB. (3)	ERYTHROBLASTOSIS	RISE OR FALL (4) IN R.B.C.	
8	1935	1/ 6– 4/24	8.0	14	—	3.2	7.3	0	—	Average for period
		1/28	12.5	15	—	2.6	6.2	0	+	Mild exacerbation
		2/12	6.4	8	0	3.6	8.9	0	+	Remission
		2/19	12.0	14	10	2.9	6.8	0	+	Exacerbation
		3/3	7.4	9	10	3.6	7.8	0	+	Remission
		3/8	11.0	12	10	2.7	6.7	0	+	
9		6/21	4.2	20	10	2.8	6.3	±	+	Mild exacerbation
10	1934–35	11/10– 2/25	7.5	17	—	3.2	6.4	+	—	Average early period
		2/25– 5/4	3.0	15	—	3.6	8.5	±	—	Average later period
		12/23	9.5	25	6	3.2	6.3	+	0	
11		10/10–11/28	12.0	22	—	1.9	6.3	0	—	Average
		11/20	15	30	10	1.8	5.9	0	0	
		12/2	5.9	23	11	2.2	7.7	0	+	

erythroblastosis, there have been a few cases in which erythroblasts have not been mentioned.

In Chart 2 the red cells are plotted against urobilin excretion. There seems to be some indication that severe erythroblastosis tends to be associated with lower red cell values irrespective of urobilin excretion, but this is not very striking since there are only a few observations in which severe erythroblastosis was not associated with relatively high urobilin excretion.

From these two charts it is evident that erythroblastosis, while often associated with greatly increased haemolysis, is not strictly dependent on the rate of blood destruction, but constitutes a third variable and is an expression of a pathological process which affects the maturation of red cells, and by so doing disturbs the ability of the erythropoietic tissue to respond effectively to the need for red cell replacement.

There are then three variables: (1) Rate of blood destruction; (2) Rate of blood formation, and (3) Tendency to immaturity considered as an expression of a disturbance in erythropoietic function. From a

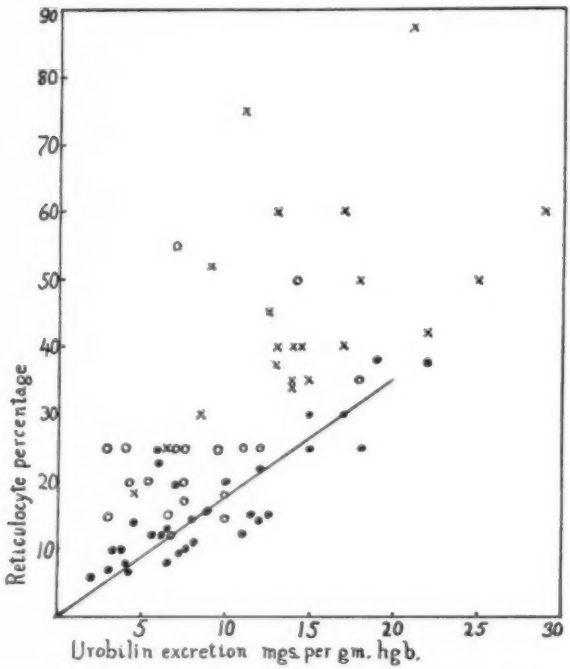

CHART 1. RELATION OF UROBILIN EXCRETION AND RETICULOCYTE PERCENTAGE

The figures from which the chart was constructed are taken from Table 1, columns 2 and 3. The diagonal line represents a theoretical normal relationship between reticulocytes and urobilin excretion on the basis of former work (see text). This chart can be used to support either the hypothesis that urobilin excretion is a measure of blood destruction or that in the absence of erythroblastosis the reticulocytes are a measure of the rate of formation, depending on which of these two propositions is assumed to be true to begin with.

Explanation of symbols: Solid circles represent cases without nucleated red cells. Hollow circles represent cases with moderate numbers of nucleated red cells, (up to 2000 per cu.m.m.). Crosses represent cases with nucleated red cells exceeding 2000 per cu. mm.

consideration of Chart 1, it is evident that compensatory regeneration as measured by reticulocyte percentage in the absence of erythroblastosis is dependent on the rate of blood destruction as measured by

urobilin excretion, not only in quiescent periods, but also, when due allowance is made for the "lag" in erythropoietic activity, during rapid rises and falls in the rate of blood destruction. We may therefore characterize these cases on a basis of blood destruction and erythroblastosis, both of which play their part more or less independently in the production of the hematological picture. Generally speaking in congenital hemolytic jaundice the disturbance giving rise to erythroblastosis is seen only in exacerbations and in infancy, though of course there are exceptions to any such statement as this. In sickle cell

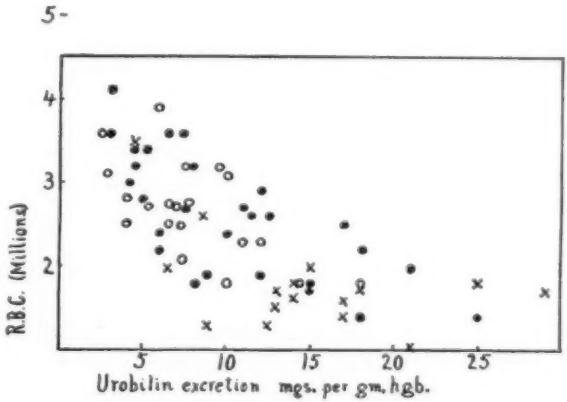

CHART 2. RELATION OF RED CELL LEVEL TO UROBILIN EXCRETION

The figures are taken predominantly from Table 1, columns 2 and 5. A few were taken from the tables representing the individual cases, where this was possible, in order to fill in portions of the chart unrepresented in Table 1.

anemia, on the other hand, erythroblastosis is much more frequently present and may reach extreme degrees in exacerbations. From the study of these cases, I believe that the presence of erythroblastosis is evidence of a disturbance in erythropoietic function and not merely a response on the part of the bone marrow to a greatly increased need for blood formation, though the latter process may well be a factor in the total situation. This erythropoietic disturbance is as much a part of the disease as is the blood destruction, and in some cases may possibly so dominate the picture that the presence of blood destruction

is hardly considered, and the case be designated "erythroblastic anemia." This attempt to consider the "hemolytic" and "erythroblastic" anemias in one group, in which individual members may differ in the preponderance of one or the other process, is at variance with the usually accepted systems of classification. This will be considered in more detail in a later paper of this series.

It was said that in the absence of erythroblastosis compensatory regeneration tended to parallel the rate of blood destruction if due allowance be made for the "lag." The principal exceptions to this general rule are found in those cases in which there seems to be a diminished erythropoietic response, or none at all. Such a hypoplastic process is occasionally seen in congenital hemolytic jaundice and is illustrated in the case of F. L. One may, of course, consider this a case in which the lag in erythropoietic activity is greater than usual, but this does not alter the fact that some degree of "hypoplasia" is present, even though it may be only temporary.

EQUILIBRIUM AND ITS DISTURBANCES

In both sickle cell anaemia and congenital haemolytic jaundice, there tends to develop a condition of equilibrium so that the disease appears to be stationary often over long periods of time. Actually there are continually occurring minor fluctuations that are sufficiently easily compensated for, so that the level of red cells and haemoglobin undergoes only minor variations. The stability of this equilibrium is greatest in congenital haemolytic jaundice in which over long periods the red cells and haemoglobin may remain at a relatively high level with little disturbance to the patient's general condition. In sickle cell anaemia, on the other hand, the equilibrium is not only more unstable but, such as it is, is established at a lower level of haemoglobin and red cells so that these patients are less able to carry on their normal life. In Mediterranean anaemia equilibrium can hardly be said to exist and the patients rarely reach puberty. The factors concerned in the development and maintenance of "equilibrium" are not known. It is not unlikely that, as the disease becomes established, factors of defense are developed to keep the balance; for often the disease is most severe and fluctuations are most violent at the onset, while later these violent fluctuations are seen with less fre-

quency. Associated with these periods before "equilibrium" has been reached, or when it has been temporarily disturbed in a crisis,

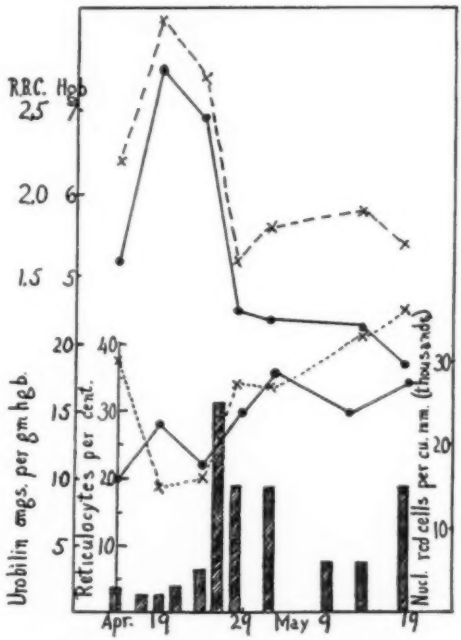

CHART 3. CHART OF D. C. AT TIME OF FIRST ADMISSION SHOWING PRINCIPALLY THE INFLUENCE OF ERYTHROBLASTOSIS AND THE VIOLENT FLUCTUATIONS IN RED CELLS AND HAEMOGLOBIN ASSOCIATED WITH A PERIOD OF EXACERBATION

The abrupt fall in red cells and haemoglobin after April 19 is associated with a return of erythroblastosis; the rise in urobilin excretion was scarcely sufficient by itself to have produced such a crisis.

R. B. C. = broken line in upper portion of chart. Hgb. = continuous line in upper portion of chart. Urobilin excretion = continuous line in lower portion of chart. Reticulocytes = broken line in lower portion of chart. Nucleated red cells = shaded columns.

is erythroblastosis, the outward manifestation of an underlying disturbance in erythropoiesis. But whether this disturbance in erythropoiesis is cause or effect cannot at present be said.

CRISES

There are three types of crisis available for study: (1) that occurring in the congenital types of haemolytic anaemia; (2) that occurring in a previously healthy individual as a result of some minor infection (Lederer's type); (3) favism, a form of haemolytic crisis apparently associated with allergy to the fava bean and even to the flower.

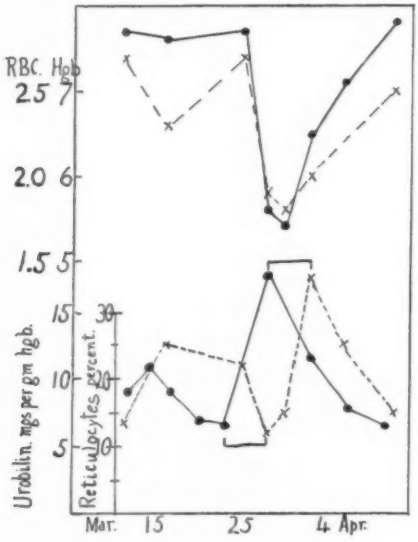

CHART 4

This is a good example of what happens in a crisis (E. C., 1935) as described in the text. The lag in reticulocyte response is well shown. The erythroblastosis that was present in this case is not charted. The crisis followed an injection of lecithin intravenously.

The course of events can be studied from the cases here reported. The first thing that happens is an abrupt increase in the rate of haemolysis reaching a peak in a few days. Usually the reticulocytes do not rise immediately but only after the lapse of several days (three to ten) (see Table 1). During the period of uncompensated haemolysis the red cells and haemoglobin may fall with alarming rapidity (for example, see Chart 4). Haemoglobinuria, however, almost never

occurs, and it is relatively rare that the red cells and haemoglobin fall so low as to threaten life. As the reticulocytes begin to rise, the red cells fall less rapidly and soon reach a level from which recovery begins. As long as the factor causing the crisis is still present, recovery is slow or does not take place, but as soon as this factor is no longer effective the rate of blood destruction falls. Blood formation now becomes

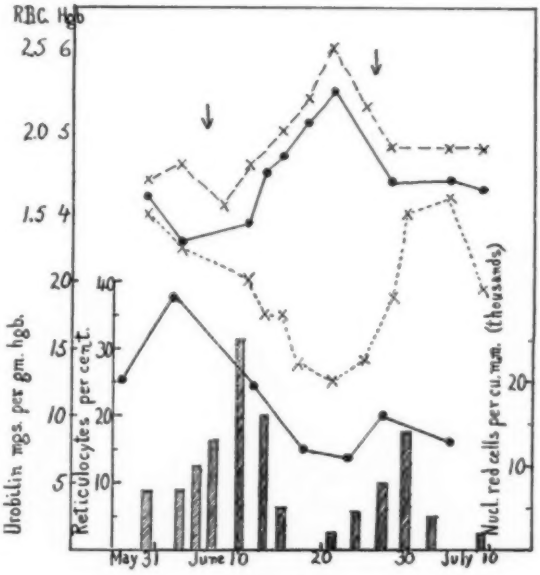

CHART 5

In this case (D. C.) the remission was induced therapeutically. The influence of erythroblastosis is well shown. The fall in reticulocytes with diminution in numbers of nucleated red cells and the later rise with increase in erythroblastosis should be noted. The arrows indicate the injection of a plasma extract to be described in the next paper.

relatively unopposed and the red cells and haemoglobin rise, often with surprising rapidity.

In the presence of erythroblastosis the course is not so simple. The reticulocytes rise to a higher point and their rise is not necessarily evidence of a compensatory rise in blood formation. In fact in one or two instances when a remission was accompanied by a diminution

in the numbers of erythroblasts, recovery was initiated by a fall in reticulocytes with a subsequent rise as the erythroblastosis reasserted itself (Chart 5). In other instances a crisis was ushered in by an increase in erythroblasts while increase in the rate of blood destruction was relatively minor (Chart 3).

In the acute case occurring in a previously healthy individual, the crisis follows very much the same course and appears to be of the same nature. The chief differences are quantitative: (1) haemoglobinuria is more likely to occur; (2) the crisis is often more severe and may be more prolonged; (3) erythroblastosis occurs with great regularity at the height of the anaemia and especially at the point of beginning recovery, and the immaturity of the erythroblasts is likely to be greater, though the numbers are less, than in the cases reported here; (4) extreme leukocytosis may occur, counts above 50,000 being reported (14); (5) the delay in reticulocyte response where the data allow it to be ascertained is somewhat longer (15). A similar prolonged lag in reticulocyte response has also been noted in congenital haemolytic jaundice especially in an acute onset of the active stage (Scott, Murray-Lyon) (see also case 2, 1932, and case 12, 1932).

The reason for the greater severity of the acute haemolytic anaemia, which is quite analogous to the greater severity of the initial crisis of congenital haemolytic jaundice or of sickle cell anaemia must be sought either in a greater severity of the haemolytic process or in a slower compensatory response of the haemopoietic tissue. The presence of haemoglobinuria suggests a higher rate of haemolysis in the acute case, but determinations of the rate of blood destruction are lacking. In this series excretion of urobilin in case 13 was relatively lower than in the cases of sickle cell anaemia or congenital haemolytic jaundice.

The lag in reticulocytes appears to offer a possible explanation. As has been said, in the majority of the congenital cases there is a lag of about three to ten days, and it has been suggested that the fall in red cells and haemoglobin was at least in part dependent on the presence of such a lag leading to a period of uncompensated blood destruction. There were a few crises in which this lag did not occur; in these there should therefore be little or no fall in haemoglobin (Chart 6). Unfortunately, the absence of a delay in reticulocyte response appears to be quite definitely associated with erythroblas-

tosis which abolishes the nicely ordered relations between urobilin excretion, reticulocyte percentage and fluctuations in red cells.

In the acute cases the lag is from about two weeks to thirty or even forty days. The reason for the greater lag is not clear. There may

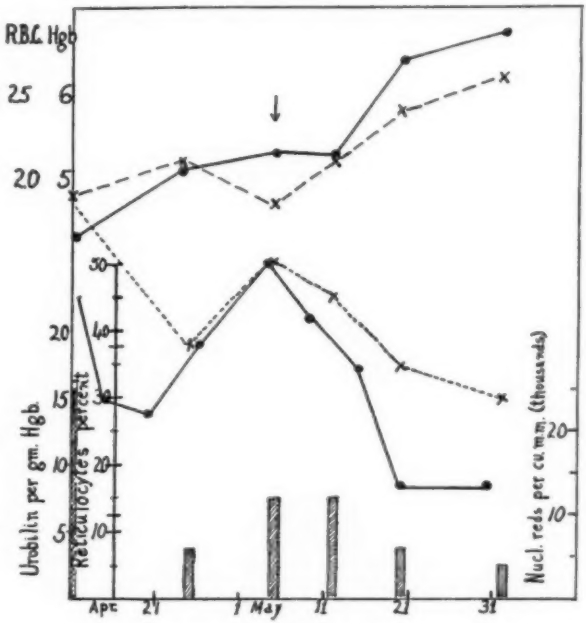

CHART 6

(E. C., 1933. An illustration of a period in which there was no reticulocyte lag during an exacerbation of haemolysis. As may be seen, there was no appreciable fall in red cells. In most of such periods the marked fluctuation in erythroblastosis renders the interpretation difficult. In this instance the course of events is what should be theoretically expected. The arrow points to the injection of a plasma concentrate to be described in the next paper.

be a factor of preparation in the congenital cases, *i. e.* the bone marrow is already in a state of such activity that an increase in activity occurs fairly promptly, while in the erythroblastic cases there tends to be no delay at all. On the other hand, in favism (18), in which the factor of preparation is lacking, there is no evidence of undue delay in

haemopoietic response. Some other factor therefore must be present. We find it in the evidence of "hypoplasia" which may be well marked in some even to the existence of a "pernicious" picture (19), and some of these acute cases have actually been reported under the designation "acute pernicious anaemia." The presence of this "hypoplastic" factor, as well as the fact that the erythroblasts often tend to be more immature than in the congenital cases, indicate a more severe haemopoietic injury which probably is a factor in the greater severity of the crisis in acute haemolytic anaemia.

SUMMARY

A number of cases of haemolytic anaemia have been studied in order to determine whether it was possible to express the relation between blood formation and destruction in the form of an equilibrium, using the reticulocyte percentage as a measure of formation and urobilin excretion as a measure of destruction. It was found that this could be done with reasonable accuracy in the absence of any considerable degree of erythroblastosis, if the urobilin excretion was expressed in relation to the total circulating haemoglobin. It is felt that the success of this attempt could be considered as evidence that urobilin excretion can be used as an index of blood destruction in the haemolytic anaemias, but that it is unwise to generalize with regard to other types of anaemia especially those in which the disturbance in haemopoiesis is at the level giving rise to "pernicious" pictures.

From the study of these cases it has become increasingly evident that pathogenically it is not possible to separate the haemolytic from erythroblastic cases, but that it is best to consider the entire haemolytic-erythroblastic group together, evaluating the relative importance of one or the other process in each individual case or at different times in the same case. This does not mean that there may not be a certain amount of distinction between different clinical types, in that, for instance, congenital haemolytic jaundice is predominantly haemolytic with comparatively little tendency to erythroblastosis except in infancy and during crises, while Mediterranean anaemia is predominantly erythroblastic, with sickle cell anaemia occupying an indeterminate position between the two.

Finally, the method of balancing blood formation against blood destruction has been used to study the course of haemolytic anaemia,

especially that of the crises. It was found that following any sudden increase in the rate of haemolysis there was usually a delay in a compensatory increase in erythropoietic activity, and that this delay was probably one important factor in the severity and duration of the crisis. From an analysis of the cases reported here, and from those in the literature, there appear to be two factors influencing the rapidity with which increased activity occurs:—(1) antecedent activity as seen particularly in the congenital cases, which tends to shorten the period of delay; (2) haemopoietic disturbance which, if sufficiently deep, may lengthen the period or even prevent any response.

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CASE HISTORIES AND TABLES

The histories that follow are brief characterizations of the cases studied, with no attempt at detail. The tables contain only the data necessary for a study of blood destruction and compensatory regeneration, under the conditions encountered during the observation of the patients. A few explanations are necessary.

As explained earlier, the reticulocyte counts are approximate. The decimal point in a reticulocyte count is meaningless and represents a complete waste of time. *Nucleated red cells* were counted against the total red cells in a stained smear. The meanings of the symbols are as follows:

± up to 1000 nucleated red cells per cu. m.m.
+ 1000–5000 nucleated red cells per cu. m.m.
++ 5000–20,000 nucleated red cells per cu. m.m.
+++ 20,000–50,000 nucleated red cells per cu. m.m.
++++ above 50,000 nucleated red cells per cu. m.m.

Total hemoglobin is calculated from the weight and hemoglobin percentage on the assumption that blood volume = 7.6% of the weight. This method is of course open to objection, but by this method we can obtain some idea of total hemoglobin.

The mgs. of urobilin excretion per gm. of hemoglobin give an idea of the severity of the hemolytic process and allow a comparison between cases in which weight varies markedly, as well as the possibility of balancing blood destruction against blood formation as explained in the body of the paper.

The notes in the final column are concerned with therapeutic attempts to be considered in the next section which appears as a separate paper following this one.

Case 1. (Table 2.) E. A., Col., B. 4/2/19. Sickle cell anaemia.

The onset of the condition can not be ascertained exactly but probably occurred sometime about the end of the second year when the child began to complain of the attacks of abdominal pain that have been so characteristic of the disease right up to the present. When first seen at 4½ years of age the patient had the disease in a very severe form, with erythroblastosis. Since that time there has been improvement, especially in a lessening of the severity of the crises, so that now they no longer threaten life, but equilibrium, though relatively stable, has been established at such a low level of red cells and haemoglobin that the boy is severely handicapped. The observations recorded here were obtained during quiescent periods and during attempts at therapy which will be discussed in a subsequent paper.

Comment. The chief interest lies in the very low red cell and haemoglobin level maintained throughout in spite of urobilin excretion that is not excessive, as urobilin excretion in sickle cell anaemia goes. In this series of cases such a low red cell level is exceptional but only a few cases at this age have been studied. R. Wr. (case 10) was similar in having low red cell level, but J. N. (case 9) had established a far better level. More cases must be studied at this age before any generalization can be established.

Case 2. (Table 3.) J. C., B. 8/27/27. Congenital haemolytic jaundice.

The signs of congenital haemolytic jaundice were first present in the first year of life, (anaemia with reduction in red cells, marked anisocytosis, polychromato-

TABLE 2

NO. OF CASE	YEAR	DATE	WEIGHT, KGS.	BLOOD					UROBILIN EXCRETION			
				R.B.C., millions	Hgb., gms.	Reticulocytes, per cent	Nucleated red cells	Total hgb., gms.	Date	Mgs. per day	Mgs. per gm. hgb.	
E. A.	1932							(125)	10/26–31	1,100	8.8	
1		11/7	30	1.7	5.5	10	0	125	10/31–11/6	1,150	9.2	Plasma intramusc. 11/6
		11/12		1.8	5.7	10	0	131	11/ 9–13	700	5.3	
		11/16	30.5	1.7	5.9	12	0	137	11/13–19	950	6.9	
		11/22		1.8	6.1	12	0	143	11/19–26	970	6.8	
		11/29	31	1.9	5.5	12	0	130	11/26–30	1,300	10.0	
		12/5		1.8	5.9	10	0	140	11/30–12/5	1,740	12.5	Plasma intramusc. 12/5
								(148)	12/ 5– 9	1,100	7.4	
		12/11	31.5	1.9	6.5	12		156	12/11–15	1,280	8.2	
		12/20		2.1	6.7	16		162	12/15–21	1,400	8.7	
	1933	10/19	32	1.6	7.0		0	170	10/29–26	1,700	10	
								(170)	10/26–11/1	1,850	11	
								(170)	11/ 1– 8	1,720	10	Plasma concentrate 11/8
		11/9		1.7	6.9	22	0	168	11/ 8–14	1,900	11.5	
		11/16		1.8	6.1	15	0	149	11/14–20	1,400	9.4	
		11/20		1.9	7.6	15	0	185				
		11/25		1.9	7.9	20	0	192	11/20–26	1,500	8.0	Plasma concentrate 11/26
		—		—	—	—		(180)	11/26–12/3	1,100	6.1	
		12/5		2.2	6.9	15	0	168	12/ 3– 9	1,220	7.3	
		12/9		2.3	7.7	12	0					
		12.13		2.4	8.6	18	0	210	12/ 9–15	1,330	6.4	
		12/19		2.2	8.2	17	0	200	12/15–19	1,450	7.3	
		12/23		2.0	6.7	18	0	163	12/19–23	1,650	10.0	
		12/30		1.8	6.3	20	0		—	—	—	

philia, a few nucleated red cells, splenomegaly), but because of the absence of jaundice the disease remained unsuspected until after the first severe crisis at about the age of five years. It was diagnosed then after the finding of greatly increased urobilin excretion in the stools. Up to the time of the first crisis the

TABLE 3

CASE NO.	YEAR	DATE	WEIGHT, KGS.	BLOOD					UROBILIN EXCRETION			
				R.B.C., millions	Hgb., gms.	Reticulocytes, per cent	Normoblasts	Total hgb., gms.	Date	Mgs. per day, average	Mgs. per gm. hgb.	
J. C. 2	1932	5/26	16	1.1	2.8	3	0	—	—	—	—	W.B.C. 3,800. Transfusion 5/30
		6/4		2.2	4.8	—	0	—	—	—	—	
		6/11		2.5	6.5	5	0	79	6/ 7–11	140	1.8	
		6/18		2.5	7.0	9	0	85	6/13–19	260	3.1	
		6/25		3.1	6.2	12	0	75	6/19–25	330	4.4	
		—	16	—	—	—	—	(75)	6/25–28	500	(6.6)	
	1933	9/28	20	2.3	—	—	—	—	9/29–10/1	1,300	(11.1)	
		10/2		2.8	7.8	13	0	119	10/ 1–5	1,350	11.5	
		10/7		2.7	7.0	15	0	106	10/ 5–11	1,300	12	Injection
		10/11		2.8	7.4	15	0	113	10/11–15	900	8	
		10/16		2.9	7.7	16	0	117	10/ 5–19	950	8.1	
		—		—	—	—	—	(135)	10/19–22	600	4.5	
		10/24		3.2	8.9	15	0	—	—	—	—	
		11/10	20	3.3	9.9	12	0	—	—	—	—	
	1934	1/6	20	3.2	9.3	—	0	—	—	—	—	Vaccination
		1/13		2.3	6.3	12	0					
		1/15		1.7	5.6	14	0	(100)	1/13–17	1,500	15	
		1/18		2.4	7.5	25	0	114	1/17–20	1,250	13	
		1/22		2.7	7.9	25	0	120	1/20–24	900	7.5	
		1/27		1.4	4.4	10	0	67	1/26–31	1,200	18	
		1/29		1.6	4.5	11	0	69	—	—	—	
		2/3		3.0	7.8	18	0	118	1/31– 2/4	850	6.1	
		2/6		3.2	9.3	25	0	141	2/ 4–10	1,700	12	
		2/9		3.4	9.7	—	0	147	—	—	—	
		2/12		2.5	7.7	20	0	117	2/12–15	2,000	17	
		2/17		2.9	8.4	17	0	128	2/15–18	1,750	14	
		2/23		2.6	7.5	30	0	114	2/18–25	1,800	16	
		3/1		3.1	8.7	25	0	132	2/25– 3/2	700	5.2	
		3/6		2.9	9.7	22	0	147	3/ 6– 9	670	4.6	
		3/10		3.2	11.3	20	0	172	9–13	900	5.2	
		3/13		3.4	10.9	20	0	—	—	—	—	
		.....		...	...	...	...	...	.....	...	...	Splenectomy, 3/13
		3/19		3.8	14.0	0	0	213	20–24	110	.52	
		3/28		3.9	13.6	occas.	0	—	—	—	—	
		4/28		4.3	14.0	occas.	0	213	4/25–28	80	.38	

disease had remained more or less quiescent, and the crisis gave the impression of an acute onset. A somewhat unusual feature was the slowness of reticulocyte response. Following this crisis the disease remained stationary for about a year and three-quarters, when following vaccination another severe crisis developed. From that time on the state of equilibrium, which up to then had been fairly satisfactory, appeared to be completely destroyed and it was with some difficulty and with frequent setbacks that the red cells were raised to over 3.5 million. Splenectomy was done and for two years the child has maintained his red cells and haemoglobin at an essentially normal level. The first period of study was during a quiescent period, the second during the crisis or series of crises that were terminated by splenectomy.

The case demonstrates the relation of the red cell and haemoglobin curves to the fluctuations in urobilin excretion, and the lag in reticulocytes following increases in urobilin excretion. A point worth mention was that, in the first year of life, ordinary dietetic measures led to considerable improvement. The same thing was encountered in other cases—F. L., R. R. and in a sister of J. C.

Case 3. (Table 4.) E. C., Col., B. 11/6/31. Sickle cell anaemia.

The onset in this case can be very exactly placed at 13 months and the first period of study commences a few days later. The onset was with a severe crisis accompanied by erythroblastosis. During the first four months the course was stormy as may be seen from the table and charts, then a more stable equilibrium appears to have been reached and since that first year crises have been much less frequent or severe. The tendency to erythroblastosis has become progressively less during a period of observation extending over four years; normoblasts in considerable numbers still appear during a crisis though absent in the quiescent periods.

Comment. This is an excellent example of a case with a marked erythroblastic component, especially in the early course. The period of study in 1935 included the complete study of an isolated crisis in which balance between urobilin and reticulocytes, lag in reticulocyte response, and fluctuations in red cells according to the preponderance of formation or destruction are well shown.

Case 4. D. C., B. 11/18/32. Sickle cell anaemia.

The child was apparently well up to her first admission to the hospital at sixteen months of age, when she had a crisis associated with the onset of pyelitis. It is probable that this crisis marked the onset of the active stage of the sickle cell anaemia as the child had been well up to that time.

The course was severe at first and was marked by numerous crises associated with marked erythroblastosis. After this initial period of instability, lasting about 6 months, the patient developed a more stable equilibrium, in which there have been no crises severe enough to necessitate special attention.

The table has been omitted to save space. A part of the course that illustrates the effect of erythroblastosis is contained in chart 3.

Case 5. F. F., white, age 9 years. Congenital haemolytic jaundice.

This boy's case was a rather unusual example of congenital haemolytic jaundice in which there developed at one time erythroblastosis. He is to be the subject of a special report to appear soon.

TABLE 4

CASE	YEAR	DATE	WEIGHT, KG.	BLOOD					UROBILIN EXCRETION			
				R.B.C., millions	Hgb., gms.	Reticulocytes, per cent	Nucl. red cells	Total hgb., gms.	Date	Mgs. per day, average	Mgs. per gm. hgb.	
E. C.	1932	12/24	7.8	2.6	6.4	25	+	38	12/21–24	205	5.3	Transfusion
								(38)	12/24–29	110	2.9	12/18/32
	1933	1/2	7.8	3.1	6.9	25	++	42	12/29– 1/4	205	4.9	
		1/10	7.9	1.7	4.3	23	++	26				
		1/14	7.9	1.7	4.5	30	++	27	1/12–16	350	13	
		1/17	7.9	1.9	4.9	60	++	30	1/17–21	340	11	
		1/23	8.0	1.9	5.1	50	++	31	1/21–27	230	7.4	
		2/2	8.1	2.3	5.0	40	+	31	1/28– 2/3	420	13	
		2/7	8.2	1.9	4.6	40	+	28	2/ 4–10	480	17	
		2/14	8.0	2.3	5.3	25	±	32	2/10–17	360	11	
		2/21	7.9	1.6	4.2	60	++	26	2/17–24	430	17	
		2/23	7.9	1.9	5.0	40	++	30				
		2/27	8.0	2.3	4.9	35	+	30	2/24– 3/3	365	12	
		3/10	8.0	2.2	5.1	25	±	31	3/ 3–10	370	12	
								(31)	3/10–16	640	21	
		3/17	8.0	1.7	5.3	40	+	32	3/16–23	360	11	
		3/28	8.0	2.4	5.8	30		35	3/23–29	500	14	
		4/4	8.0	1.9	3.9	45	++	24	3/29– 4/5	450	19	
		4/11	8.0	2.7	4.3	60	+++	26	4/ 5–11	750	29	Transfusion
								(28)	4/11–18	425	15	4/11
		—						(30)	4/18–22	425	14	
		4/23	8.0	2.1	5.1	—	—	31	4/22–28	600	19	
		5/5	8.0	1.8	5.3	50	++	32	5/ 1– 6	810	25	Plasma concen-
		5/12	8.0	2.1	5.3	45	++	32	5/ 6–12	670	21	trate 5/5
								(36)	5/12–18	600	17	
		5/20	8.1	2.4	6.4	35	++	40	5/18–22	340	8.5	
		6/1	8.2	2.6	6.6	30	++	41	5/26– 6/2	350	8.5	
		6/8	8.4	2.5	6.1	—	++	39	6/ 2–10	400	10	Plasma concen-
		6/16	8.6	2.7	7.1	40	++	46	6/11–17	380	8.3	trate 6/10
		6/22	8.8	2.7	6.9	25	+	46	6/17–23	270	5.9	
		6/26	8.8	2.7	6.8	20	—	45	6/23–29	240	5.3	
		7/1	8.9	2.6	6.3	20	—	43	6/29– 7/6	280	6.5	Plasma concen-
		7/5	8.9	3.1	7.8	—	++	53	7/ 6–10	260	4.9	trate 6/29
		7/14	9.0	3.0	7.1	18		49	7/10–15	170	3.5	

TABLE 4—*Concluded*

CASE	YEAR	DATE	WEIGHT, KG.	BLOOD					UROBILIN EXCRETION			
				R.B.C., millions	Hgb., gms.	Reticulocytes, per cent	Nucl. red cells	Total hgb., gms.	Date	Mgs. per day, average	Mgs. per gm. hgb.	
E.C.	1935	2/5	13.0	—	8.1	12			—	—	—	
		2/10	13.1	—	5.9	—		59	2/ 9–12	850	14.4	
		2/16	13.2	3.0	6.7	20	+	67	2/13–19	450	6.7	
								(67)	2/19–23	550	8.1	
		2/25	13.5	2.5	6.6	22	+	68	—	—	—	2/23 Plasma extract; ether soluble fraction
		3/2	13.7	2.7	8.1	20	+	84	3/ 1– 8	540	6.6	
		3/11	14	2.7	7.7	22	+	82	3/ 8–13	730	8.9	
		3/16	14	2.3	7.6	30	+	81	3/13–18	820	10.1	
		3/25	14	2.7	7.7	22	+	82	3/18–25	540	6.6	
		3/28	14	1.9	5.6	12	±	60	3/25–31	1,075	18	3/25 Injection of lecithin
		3/30	14	1.8	5.4	15	+	57				
		4/2		2.0	6.5	35	+	69	3/31– 4/4	650	11.5	
		4/6		—	7.1	25	+	75	4/ 4– 9	600	8.0	
		4/12	14	2.5	7.8	15	±	83	4/10–12	540	6.5	

Case 6. D. H., Col., B. 8/6/25. Sickle cell anaemia.

It is difficult to say when the active phase commenced. She was brought to the hospital at one year of age with a haemoglobin of 40% and splenomegaly, but no studies were made to determine the nature of the anaemia. At five years of age, when the diagnosis was made, the disease was already well established. The study reported here was undertaken during her prolonged stay in the hospital following the diagnosis.

An outstanding feature of the course in this case is the high degree of erythroblastosis that accompanied the crises, even when the increase in urobilin excretion was insignificant, whereas between crises there was scarcely a nucleated red cell to be found.

Case 7. J. N., Col., B. 2/8/19. Sickle cell anaemia.

Nothing is known of the time of onset. The diagnosis was made at the time the child was nine years of age when she was admitted to the hospital with a diagnosis of rheumatic endocarditis. Since that time equilibrium has been established at a level of about 3 million red cells, but is disturbed by occasional crises. It was during one of these crises that she was admitted for the study recorded here.

Comment. The crisis on admission was accompanied by marked erythroblastosis with its usual effect on the reticulocytes. After a rapid recovery there was a second mild exacerbation. In this instance the reticulocytes rose along with the urobilin excretion and there was no fall in haemoglobin until later.

Case 8. R. R., white, B. 2/20/34. Congenital haemolytic jaundice.

The disease in this case may have been present at birth if a history of severe jaundice during the first two weeks of life, and pallor that persisted is to be considered evidence. At the age of five months the diagnosis was definitely established in spite of the absence of definite jaundice. At this time red cells numbered 1.7 million, haemoglobin was 32 per cent, and there were present many nucleated red cells. With ordinary dietary measures including administration of iron and liver extract the haemoglobin rose at first to as high as 74% but did not maintain this level. A severe crisis occurred with return of erythroblastosis following which equilibrium was reached at a haemoglobin level of about 50% haemoglobin. The period represented by the study, the details of which are not reported here, was one of quiescence disturbed only by attempts at therapy to be described in a subsequent paper. Nucleated red cells were occasionally seen but during this period never became a factor in the situation.

Comment. This is one of the few cases in which the evidence is fairly convincing that the disease was present at birth. The marked improvement that occurred at first with ordinary dietary and medicinal measures is not unusual, since it was encountered in both J. C. (case 1) and F. L. (case 12) in this series and also occurred in a sister of J. C. who also has the disease. The presence of erythroblastosis which is characteristic of the onset of congenital haemolytic jaundice in infancy was present at first but tended to become less marked as equilibrium was established.

Case 9. E. T., Col., B. 11/2/27. Sickle cell anaemia.

When first seen at the age of three years the disease was probably already present in active form but was undiagnosed until the following year. He had attacks every few months initiated by abdominal pain and vomiting. It was in one of these that he was admitted to the hospital for the period of study reported here. He was soon taken out again and has not been seen since.

Case 10. (Table 5.) R. W., Col., B. 3/15/33. Sickle cell anaemia.

The onset of the active phase occurred apparently at about one year of age with pain and swelling in the joints and cardiac enlargement, but it was not until six months later that the condition was recognized and the blood studied. There was apparently at no time a crisis that forced attention on the condition of the blood, and the admission to the hospital was because of the development of pyelitis. There were erythroblasts in considerable number on admission in September, 1934 (2000 per cu. mm.) but these soon largely disappeared and were present thereafter only in small numbers.

Comment. The course in this child was very mild compared to that in E. C. (case 3) or D. C. (case 4). In fact the chief interest lies in the fact that equilibrium was well preserved throughout and in this respect the contrast with the other two is striking.

Case 11. R. Wr., Col., B. 2/22/23. Sickle cell anaemia.

At the time of the period of study recorded here the child was nearly eleven

years of age. He had been diagnosed as having sickle cell anaemia at five years of age, but details of the earlier history and period between five and eleven years are lacking. He had apparently established some sorts of equilibrium that permitted

TABLE 5

CASE NO.	YEAR	DATE	WEIGHT, KG.	BLOOD					UROBILIN EXCRETION			
				R.B.C., millions	Hgb., gms.	Reticulocytes, per cent	Nucl. red cells	Total hgb., gms.	Date	Mgs. per day	Mgs. per gm. hgb.	
R. W.	1934	11/10	11.0	3.0	8.4	—	+	70				
10		11/20		—	7.0	—	+	60	11/14–22	350	5.0	
		11/30	11.1	3.4	5.8	20	+	49	11/30–12/5	350	7.1	
		12/8	11.2	3.2	6.6	12	±	57	12/ 5–12	450	7.9	
		12/18	11.3	3.3	6.1	20	±	53	12/12–30	400	7.6	
		12/24	11.3	3.2	6.4	21	±	56	12/20–26	530	9.5	
		12/29	11.3	3.4	6.3	25	+	55	12/26– 1/1	510	9.3	
	1935	1/4	11.4	3.1	7.3	18	+	64	1/ 1– 7	500	7.8	
		1/11	11.4	2.9	7.2	18	±	63	1/ 7–14	520	8.3	
		1/16	11.4	3.1	7.6	15	+	68	1/14–20	430	6.3	
		1/23	11.5	3.5	7.8	15	±	69	1/20–27	430	6.2	
		1/31	11.5	3.6	8.8	25	±	79	1/27– 2/4	450	5.7	
		2/10	11.6	3.9	7.7	25	+	69	2/ 4–10	430	6.2	
		2/16	11.6	3.3	8.2	—	±	75	2/10–19	400	5.3	Plasma extract 2/19/35
		2/23	11.7	3.5	7.6	16	+	70	2/19–25	440	6.3	
		2/28	11.7	3.5	8.6	18	++	78	2/25– 3/3	350	4.5	
		3/5	11.8	3.6	9.1	18	±	81	3/ 3– 8	300	3.7	
		3/14	11.9	3.8	10.0	22	±	91	3/ 8–14	270	3.0	
		3/19	11.9	3.4	8.2	20	±	76	3/14–19	345	4.5	Plasma extract 3/19/35
		3/25	11.9	4.1	9.7	12	0	88	3/19–25	270	3.1	
		3/31	12.0	3.8	8.6	15	±	79	3/25–31	210	2.7	
		—	—	—	—	—		(81)	3/31– 4/6	310	3.8	
		4/7	12.4	3.9	8.9	22	±	82	4/ 6–12	270	3.3	
		4/13	12.4	4.1	9.0	15			—	—	—	
		4/19	12.4	3.9	8.6	15		79	4/14–20	200	2.5	
								(76)	4/20–26	225	3.0	
								(73)	4/26– 5/2	220	3.0	
		5/4	12.4	3.2	7.6	12		70	5/ 2– 7	276	3.9	

life to go on, but at a level of haemoglobin that was insufficient for normal activity. Crises were absent, but there was continued shortness of breath and cardiac hypertrophy. After discharge from the hospital he was taken to another city where he died as the result of a sudden crisis.

Comment. In this case as in that of E. A. (case 1) the principal feature is the very low red cell count compared with the level of urobilin excretion, and the fact that variations in blood destruction as measured by urobilin excretion had little influence on the red cell count, though the slight changes that were present were in accord with expectation.

Case 12. (Table 6.) F. L., white, B. 2/2/27. Congenital haemolytic jaundice.

TABLE 6

CASE	YEAR	DATE	WEIGHT, KG.	BLOOD					UROBILIN EXCRETION			
				R.B.C., millions	Hgb., gms.	Reticulocytes, per cent	Nucl.-red cells	Total hgb., gms.	Date	Mgs. per day	Mgs. per gm. hgb.	
F. L.	1932	5/20	17	1.9	4.0	occas.	0	52	5/20–23	450	8.7	
		5/24		1.8	3.5	occas.	0	45	5/23–26	380	8.4	Transfusion 5/24
		5/27		2.5	5.3	—	0	69	5/26– 6/1	180	2.6	Transfusion 5/27
		5/30		3.2	7.0	±	0	91				
		6/4		4.2	8.4	±	0	109	6/ 1– 4	70	.64	
		6/23		3.5	8.4	5	0	109	6/21–23	300	2.8	
M. Y.	1934	6/15	6.1	2.5	7.5	—	+					Immaturity marked. "Megaloblasts"
		6/20		1.7	5.9	10	+	27	6/17–20	90	3.3	
		6/22		1.6	5.9	10	±	27	6/20–23	60	2.2	
		6/27		1.8	7.3	8	±	34	6/27– 7/1	135	4.0	
		6/30		1.4	6.5	6	±	30				
		7/2		1.5	4.8	5	+	22	7/ 1– 4	130	5.9	Transfusion 7/2
		7/6	6.2	2.5	8.2	5	±	39	7/ 4– 7	200	5.1	
		7/9		2.4	8.7	10	+	41	7/ 9–12	120	2.9	
		7/16		2.6	9.7	6	±	46	7/12–15	110	2.4	
		7/21		2.8	10.5	4	0	50	7/19–22	60	1.2	

As in the case of J. C. (case 2) evidence of the disease was abundantly present in the second year of life, (splenomegaly, reduction in red cells, polychromatophilia, anisocytosis, microcytosis), but because of the absence of jaundice the disease was not suspected until after a crisis at five years that led to a determination of her urobilin excretion. Subsequently the resistance of the red cells to hypotonic salt solution was found decreased and eventually splenectomy was performed. The crisis in 1932 came at a time of quiescence. The only unusual features were the failure of reticulocyte response and a moderate leukopenia (4,400). As there was no tendency to a rise in red cells and haemoglobin over several days, a transfusion was given. The subsequent course has no special

significance for this report as the coöperation of the parents could not be obtained for any detailed study.

Comment. The interest in this case lies in the presence of signs of "hypoplasia" with delayed response of haematopoiesis, and in the fact also exemplified by J. C. (case 2) that jaundice may be absent in these cases.

Case 13. (Table 6.) M. Y., B. 10/19/33. Acute haemolytic anaemia (?).

The child was seen for the first time at eight months of age. At this time anaemia was already present, and there was nothing in the account given by the mother to indicate when the disease first became manifest. The haemoglobin and red cells fell with great rapidity and the child was admitted to the hospital. The course is represented in the charts. Whether to classify this case under congenital, haemolytic jaundice, or under acute haemolytic anaemia remains in doubt, even after two years. The condition as it appeared in this child was similar to that in other cases reported as congenital haemolytic jaundice in infancy, and the increased fragility in both the patient and his father seemed to confirm this diagnosis. On the other hand, his blood has been normal in every way since his discharge even during infections, including pneumonia, and the red cell fragility has also been repeatedly normal. His case is classed here as one of acute haemolytic anaemia, because the course exemplifies the course in the acute cases.

Comment. This case exemplifies the fact that it may be difficult to distinguish between acute haemolytic anaemia and congenital haemolytic jaundice at the onset (*cf.* Beutler and Murray Lyon). It is also an example of a case in which immaturity of red cells played a dominant rôle since the urobilin excretion was never excessively high.

STUDIES IN HAEMOLYTIC ANAEMIA

II. The Presence of an Anti-haemolytic factor in Human Plasma

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In the first part of this work were recorded observations dealing with three aspects of haemolytic anaemia which have heretofore received little attention, namely: (1) The equilibrium between blood destruction and formation as measured, respectively, by urobilin excretion in the stools and the reticulocyte count; (2) the part played by erythroblastosis considered as a result of disturbance in erythropoiesis; (3) the mechanism of the crises in terms of an acute disturbance in the equilibruim between blood destruction and formation. This second part deals with a study of the mechanism by which transfusion acts in haemolytic anaemia.

The cases used in this part are the same as those recorded in the previous one, so that there is no necessity to repeat the tables and charts.

The beneficial effect of transfusion in the haemolytic-erythroblastic group[1] is of course well known. The most dramatic results are seen in the acute cases, in which transfusion not infrequently appears to terminate the process abruptly; but even in the congenital or constitutional cases the total effect of transfusion transcends the immediate result of blood replacement. Since considerably more blood is destroyed in a day than could be replaced by transfusion, it is probable that in many cases the replacement of blood is of relatively minor importance, and that the chief effect of transfusion is to in-

[1] The use of this term was explained in the previous part. It is used because of the fact that in the cases with erythroblastosis as a predominant feature haemolysis is probably always present at least to some extent, while in those cases that could be classified as predominantly haemolytic, at least some tendency to erythroblastosis is usually present.

TABLE 1

Effect of transfusion on the urobilin excretion and red cells in cases of haemolytic anaemia

Note: The case number refers to that in the previous section which appears as a separate paper preceding this one. As explained in the previous paper the urobilin excretion is presented both as milligrams per day (upper line), and as milligrams per gram of haemoglobin (middle line). The latter value represents an index of blood destruction and is used in the construction of the charts since it is believed to give a better idea of the severity of the haemolytic process. Normally the urobilin excretion per gram of haemoglobin should lie between 0.2 and 0.4 milligram.

NAME AND NUMBER	DATE OF TRANSFUSION		DAYS BEFORE TRANSFUSION				DAYS AFTER TRANSFUSION						
			20–15	15–10	10–5	5–0	0–5	5–10	10–15	15–20	20–25	25–30	
E. C. Case 3	12/18/32	Urob.				280	205	110	205		350	340	Erythroblastosis
		R.B.C.				7.2	5.3	2.9	4.9		13	11	
							2.6		3.1		1.7	1.7	
	4/11/33	Urob.	500	450		750	425	425	600		810		Erythroblastosis
			14	19		29	15	14	19		25		
		R.B.C.		2.4	1.9	1.7			2.1		1.8		
J. C. Case 2	5/30/32	Urob.							140	260	330	500	
									1.8	3.1	4.4	6.9	
		R.B.C.				1.1	2.2		2.5	2.5		3.1	
G. F.	1/20/31	Urob.							375	540		600	
									2.6	4.5		5.0	
	4/ 2/31	Urob.		1700		1500	1000	850	500				
				16		15	7.5	6.2	3.6				
		Hgb.		4.5		4.5	5.7	6.0	8.3				

D. H. Case 6	11/ 2/30	Urob.		575	575	540	450	210		105	240		
		Hgb.				10.5	7.3	2.9		1.5	3.6	6.3	
						5.1	6.2	7.0					
	12/10/30	Urob.	240		625	800	450	350				800	
			3.6		7.9	14	6.7	5.1				11.0	
		Hgb.		6.3	5.1	5.2	6.4					7.0	
F. L. Case 12	5/24/32	Urob.				450	180	70				300	
						8.7	2.3	0.64				2.8	
		R.B.C.				1.8	3.2	4.2				3.5	
K. O.		Urob.				125	70	85					Erythroblastosis. Sepsis.
						15	5.0	7.1					
		R.B.C.				1.2		1.5					
M. Y. Case 13		Urob.				130	200	115		60			
						5.9	5.1	2.6		1.1			
		R.B.C.				1.5	2.5		2.6		3.2		
D. H. Case 6	1/22/31 Plasma. intra.-ven.	Urob.		800		1200	800	610	500	700			Chart 6, previous part
				11		19	15	9.8	6.4	9.5			
		Hgb.		7.0		5.0		5.7	7.1	6.6			

fluence in some way the processes responsible for the condition of the blood. The various theories advanced to explain this action need not be reviewed here. The observations recorded in this paper have resulted from the more or less chance finding in a case of sickle cell anaemia that a transfusion was followed by a marked fall in urobilin excretion and a subsequent return to the previous level.

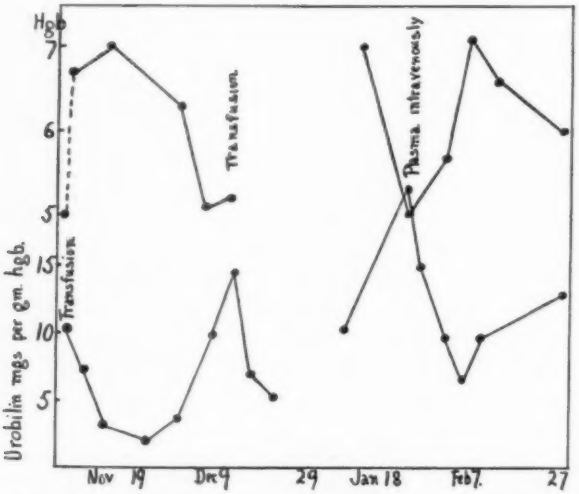

CHART 1. COURSE OF HEMOGLOBIN AND UROBILIN EXCRETION IN D. H. (TRANSFUSION AND PLASMA INTRAVENOUSLY)

Explanation: In this and subsequent charts the following symbols are used: The continuous line in the upper part denotes hemoglobin; the broken line in the upper part denotes red cells; the continuous line in the lower part denotes urobilin excretion; the broken line in the lower part denotes reticulocyte percentage; the shaded columns represent erythroblasts per cu. m.m.

Note the temporary effect of transfusion and the fact that the plasma had as marked effect in raising hemoglobin as the transfusion of whole blood.

Table 1 contains the observations on the effect of transfusion on the urobilin excretion. From these figures there is no doubt concerning the reduction of urobilin excretion, but one or two of the periods should receive special mention because they illustrate some of the difficulties of interpretation. These difficulties are: (1) Marked fluc-

tuation in the period preceding the beginning of treatment, so that it becomes impossible to exclude coincidence; (2) absence of a preliminary period of observation; (3) a complete cure of the condition, or at least a permanent reduction in urobilin excretion. The ideal conditions are a preliminary period with minimal fluctuation or steadily rising excretions, a prompt fall in urobilin excretion following the treatment and a subsequent return to the previous level within

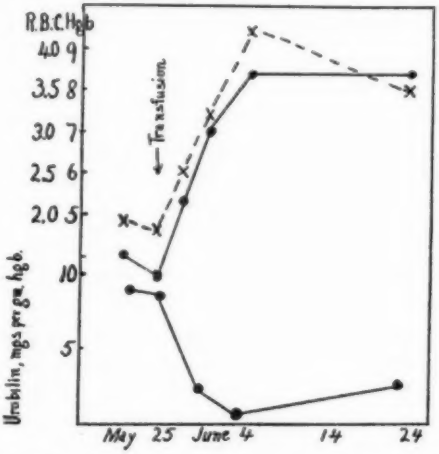

CHART 2. EFFECT OF TRANSFUSION IN CONGENITAL HEMOLYTIC JAUNDICE (F. L.)

Note: The reduction in urobilin excretion to a point practically normal for the child is unusual. The subsequent rise is typical of the constitutional hemolytic anemia and serves to distinguish it from the otherwise similar acute hemolytic anemia. In this case the rise would appear greater if the urobilin had been charted as mgs. per day rather than in relation to total hemoglobin.

the period of observation. These conditions are present in the case of E. C. (4/11/33) and D. H. (11/2/30) (12/10/30) and (1/22/31), the latter providing a continuous period of observation of over three months. In the case of J. C. and G. F., on the other hand, there was lacking any period of preliminary observation and the only fact that allows one to say that there was some effect from transfusion was the subsequent rise during the period of observation due to the termina-

TABLE 2

Effect of intramuscular injection of a protein-free concentrate of human plasma on urobilin excretion and red cells

NAME AND NUMBER	DATE OF INJECTION		DAYS BEFORE INJECTION				DAYS AFTER INJECTION						
			20–15	15–10	10–5	5–0	0–5	5–10	10–15	15–20	20–25	25–30	
E. A. Case 1	11/ 8/33	Urob.	1700		1850	1720	1900	1400		1500			
			10		11	10	11.5	9.4		8.1			
		R.B.C.	1.6			1.7		1.8		1.9			
	11/26	Urob.		1400		1500	1100		1220	1330	1450	1650	
				9.4		8.1	5.7		7.3	6.4	7.3	10	
		R.B.C.		1.8		1.9		2.2	2.3	2.4	2.0	1.8	
J. C. Case 2	10/ 9/33	Urob.		1300	1350	1300	900	950	600				
					11.5	12	8	8.1	4.5				
		R.B.C.			2.8	2.7	2.8	2.9	3.2				
	2/12/34	Urob.				1700	1850		1850				Chart 1
						12	15.5		16				
		R.B.C.				2.5	2.9		2.6				
	2/19/34	Urob.		1700		1850	1800	700	670	900			Chart 1
						15.5	16	5.2	4.6	5.2			
		R.B.C.				2.9	2.6	3.1	2.9	3.2			
	3/ 2/34	Urob.				700	670	900					
						5.2	4.6	5.2					
		R.B.C.				3.1	2.9	3.2	3.4				

E. C. Case 3	5/ 5/33	Urob.	425	600		810	670	600		340		350	Erythroblastosis. Chart 4 previous part
			14	19		25	21	17		8.5		8.5	
		R.B.C.		2.1		1.8		2.1		2.4		2.6	
	6/10/33	Urob.	340		350	400	380	270		240	280		Erythroblastosis
			8.5		8.5	10	8.3	5.9		5.3	6.5		
		R.B.C.	2.4		2.6	2.5		2.7		2.7	2.6		
	6/29/33	Urob.	380	270		240	280	260	170				Erythroblastosis
			8.3	5.9		5.3	6.5	4.9	3.5				
		R.B.C.		2.7		2.7	2.6	3.1	3.0				
E. T. Case 9	6/23/33	Urob.				350	290	210	270				
						4.2	3.6	2.2	2.7				
		R.B.C.				3.2	2.8	3.0	3.1				
R. Wr. Case 11	10/30/33	Urob.	1200	1400	1350	1550	1550	1300	1060	950	1525		
			12	13	12.5	15	15	13	11	10	15		
		R.B.C.		1.8		2.0		1.8	1.7	2.0	1.8		
	11/22/33	Urob.	1300	1060	950	1525	1550	650	800		1000	1020	
			13	11	10	15	14	5.9	7.6		7.4	7.5	
		R.B.C.	1.8	1.7	2.0	1.8	2.0	2.0	2.0		2.2	2.2	

tion of the transfusion effect. By themselves these results are of course doubtful, but taken in conjunction with other cases showing that the effect of transfusion must be expected to come to an end within three weeks they acquire some significance. This point will become plain as the next two groups of cases are considered. In the case of M. Y. we are dealing with an acute hemolytic anemia in which complete recovery took place following a transfusion. Here it is impossible to state that the transfusion was the deciding factor, for these cases are self limited. However, for a ten-day period before the transfusion was given, there had been no tendency to betterment and it is not unlikely that the transfusion hastened the recovery.

It may be seen that in the case of D. H. an injection of plasma intravenously had the same effect as transfusion. This led to the attempt to concentrate the plasma for intramuscular injection. The procedure was as follows. To plasma from freshly drawn citrated blood were added two volumes of alcohol. The precipitated proteins were separated off and the supernatant alcoholic fluid was evaporated in a vacuum to dryness. The residue was extracted with 95% alcohol. This alcoholic solution was concentrated at under 40°C., filtered to remove proteins and made up to a volume such that 1 cc. of alcoholic solution was equivalent to 20 cc. of the original blood. Two cubic centimeters of this was prepared for the injection by evaporating off the alcohol and taking up the residue in freshly distilled sterile water, using less than 1 cc. of water. The result was an emulsion that was injected directly into the buttocks of the patient.

The results of the injection of this protein-free plasma concentrate are contained in Table 2. There was in the majority of cases a definite reduction in urobilin excretion, but the effect on red cells and hemoglobin was less striking except in the cases of J. C. and E. C. In the case of E. A. there was little change in the first of the two periods, while in the second the reduction in urobilin excretion was definite and accompanied by a rise in red cells. The results in J. C. and E. C. were definitely successful both in the reduction of urobilin excretion and rise in red cells and hemoglobin, and are graphically represented in Charts 3 and 4. E. T. was admitted in a crisis and the study was incomplete, since it lacked an adequate preliminary period and was cut short by removal of the child from the hospital. As it stands by

itself it must be put down as inconclusive, but as a member of a series all showing the same tendency it has significance. In the case of R. Wr., the study was adequate in regard to preliminary periods and

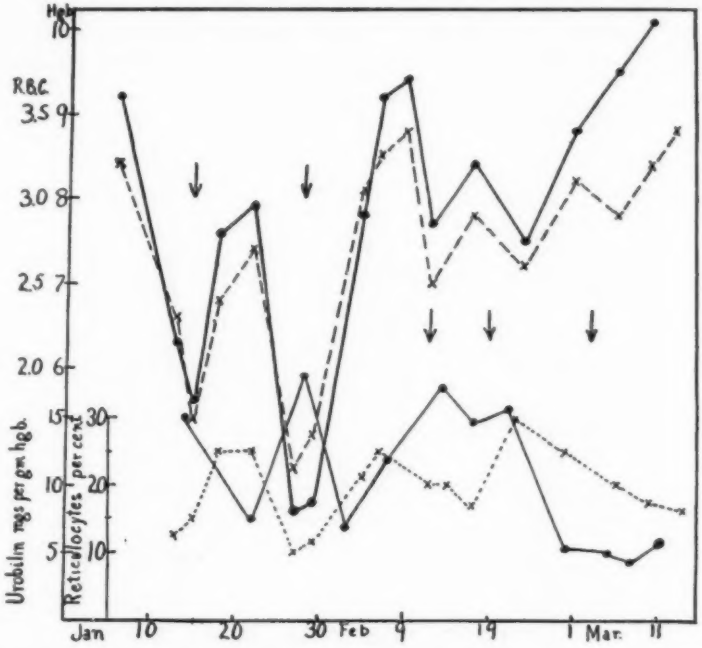

CHART 3. EFFECT OF INJECTIONS OF PLASMA CONCENTRATE IN CASE OF CONGENITAL HEMOLYTIC JAUNDICE (J. C., CASE 2)

This period was one of what might be called continuous exacerbation which started a week after vaccination. A previous injection in this child had been followed by a rise in hemoglobin lasting a month. During this period of exacerbation, as may be seen, injections every two weeks were insufficient, but when they were given at shorter intervals the red cells and hemoglobin rose satisfactorily. This is one of the best therapeutic results obtained, but could not be considered to replace splenectomy.

length of observation. In the first period the reduction in urobilin excretion took place rather slowly, and was relatively slight, in the second period the result was much clearer. In neither of these periods

nor in that of E. T. was there any appreciable change in red cells or hemoglobin.

The next step was to remove the lipoids from the concentrate by means of extraction with ether and inject the lipoid-containing and lipoid-free fractions separately. In the case of the lipoid-containing fraction, two injections were without effect, while a third was followed

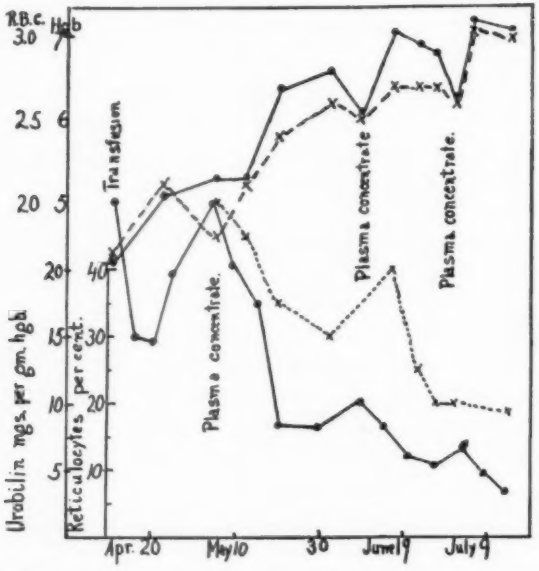

CHART 4. EFFECT OF INJECTIONS OF PLASMA CONCENTRATE IN CASE OF SICKLE CELL ANEMIA (E. C., CASE 3)

This is a very satisfactory result, but one cannot attribute it entirely to the injections of plasma concentrate. Without doubt the termination of winter infections had some influence.

by a definite rise in urobilin excretion. Table 3 contains the results following injection with the lipoid-free fraction. In the case of D. C. the first period showed a definite reduction in urobilin excretion accompanied by a rise in red cells. Since this child had been followed for a long preliminary period without the appearance of any tendency to reduction in urobilin excretion, this result has all the characteristics

of success demanded in a previous paragraph (adequate control period without fluctuations, prompt and significant fall in urobilin excretion and subsequent return to the former level of secretion). It was

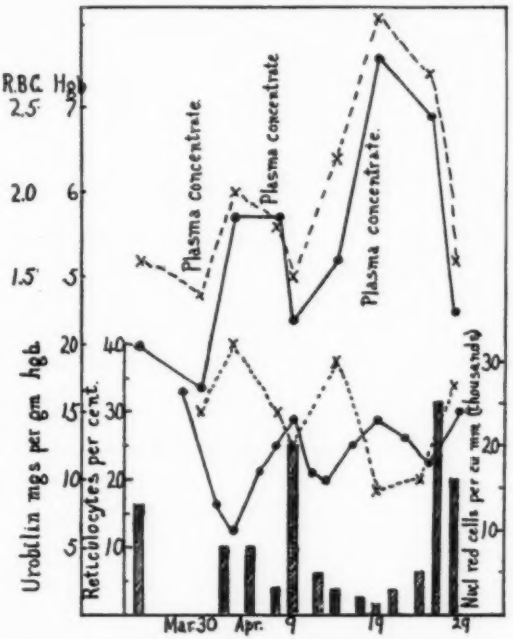

CHART 5. EFFECT OF INJECTIONS OF PLASMA CONCENTRATE IN CASE OF SICKLE CELL ANEMIA (D. C.) WITH MARKED TENDENCY TO ERYTHROBLASTOSIS

The influence of erythroblastosis in limiting the effectiveness of the injections is well illustrated. This child had pyelitis and the waves of erythroblastosis coincided with febrile exacerbations. It is therefore doubtful whether the injections had any influence on the erythroblasts, although the effect on urobilin excretion is clear. From experience in other cases, the apparent effect on reticulocytes is probably coincidence.

found impossible to repeat the results in the next two periods. It is possible, however, that in the third period longer observation would have shown a reduction in the urobilin excretion, since there was a definite rise in red cells two and one half weeks after the injection.

TABLE 3

Effect of intramuscular injection of ether-insoluble fraction of protein-free plasma concentrate on urobilin excretion and red cells

NAME AND NUMBER	DATE OF INJECTION		DAYS BEFORE INJECTION				DAYS AFTER INJECTION						
			20–15	15–10	10–5	5–0	0–5	5–10	10–15	15–20	20–25	25–30	
D. C. Case 4	6/ 6/34	Urob.	470	370		450	320		240		300		Erythroblastosis. Chart 8 of previous part
			18	13		19	12		7.5		7.9		
		R.B.C.	1.8	1.7		1.8	1.8	2.0	2.5		2.0		
	6/26/34	Urob.		240		300		250		290	370		Erythroblastosis
				7.5		7.9		8.3		10	12		
		R.B.C.		2.5		2.0		1.9		1.9			
	7/19	Urob.			290	370	410	350					Erythroblastosis
					10	12	14	12					
		R.B.C.			1.9	1.9	1.7	1.8		2.5			
F. F. Case 5	3/27/35	Urob.		850	1050	1175		725	550				
				4.7	6.4	7.4		4.0	3.1				
		R.B.C.			2.5	2.1	2.2	2.5	2.4				
	10/1/36	Urob.				1600	1200	1150		1100	840		Erythroblastosis
						15	11.5	14		14	9.8		
		R.B.C.				1.4	1.8		1.4		1.3		
	11/28/36	Urob.		1300	780	1350		950	1200		900		Erythroblastosis
				15	8.8	15		15	21		10.5		
		R.B.C.		1.0	1.0	1.1		1.0	1.0		1.5		

R. R. Case 8	1/14/35	Urob.			330	340	205	360	560		375		
		R.B.C.			9.5 3.1	8.3 3.0	4.5 3.4	7.2 3.5	12.5 3.1	2.8	6.4 3.0	3.6	
	2/21/35	Urob.		375		560	380	405		540	510		
		R.B.C.		6.4 3.0	3.6	12.0 2.9	7.6 2.9	7.4 3.6		11.0 2.7	7.3 3.5	3.4	
	3/25/35	Urob.				470		420	430		425		
		R.B.C.				8.4 3.3		8.2 3.1	7.4 3.5	3.4	7.2		
R. W. Case 10	2/19/35	Urob.	450	430	400		440	350	300	270	345		
		R.B.C.	6.7 3.6	6.2	5.3 3.9	3.3	6.3 3.5	4.5 3.5	3.7 3.6	3.0 3.8	4.5 3.4		
	3/19/35	Urob.			270	345	270	210	310	270	200	225	
		R.B.C.			3.0 3.8	4.5 3.4	3.1	2.7 4.1	3.8 3.8	3.3 3.9	2.5 4.1	3.0 3.9	

In the case of F. F. the first period showed a definitely positive result, while the next two periods were equally negative. Injections in the case of R. R. were at best doubtful mostly because the rather wide fluctuations in urobilin excretion rendered interpretation difficult, but there may have been in the first two periods a rather brief effect, In the case of R. W. the results in both periods are definitely positive, both on the urobilin excretion and the red cells.

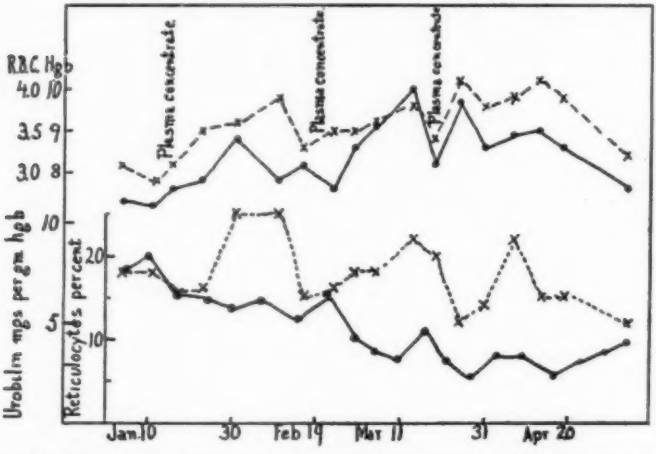

CHART 6. EFFECT OF INJECTIONS OF PLASMA CONCENTRATE (ETHER-INSOLUBLE FRACTION) IN CASE OF SICKLE CELL ANEMIA (R. W.)

In this case the condition was mild and it is therefore not so easy as in some to demonstrate any effect. Each injection, however, was followed by a demonstrable fall in urobilin excretion and rise in red cells.

As has been indicated, there are certain definitely negative results and certain other limitations to be set to the effectiveness of the injections. In the first place, the effects of transfusion and of the injections of the concentrate were limited to two or three weeks at the longest and might be much shorter. The shorter periods were encountered chiefly during the winter months and during periods of prolonged exacerbation. This is well illustrated in the case of J. C. The injection given on October 19 was followed by a rise in red cells lasting four weeks. The total duration of the reduction in urobilin

excretion could not be determined owing to the fact that the child had had to leave the hospital, but the urobilin was still falling at the end of two weeks. Later he had an exacerbation following vaccination and continued to have a tendency to exacerbation for the next three months. During this time it became necessary to give the injections at weekly intervals in order to keep the urobilin at a low point and allow a rise in red cells and hemoglobin. In the case of E. C. the injections up to May had only a very temporary effect, but after the one given on May 5, there was a steady fall in urobilin excretion and a rise in red cells with injections given about once a month.

It is not unlikely that this shortening of the time during which the transfusion or injection was effective was related to infection; and not infrequently an intercurrent infection actually terminated what up to the time promised to be a favorable result.

Infection is not the only factor, however, for the presence of erythroblastosis even in the absence of infection seems to have an unfavorable influence. This was a factor in the frequent failure in the case of D. C. in which the successful periods were associated with reduction in erythroblastosis and the unsuccessful periods with its continued presence. This is also illustrated in the case of F. F. in whom an injection in March, 1935, was followed by a well marked reduction in urobilin excretion and rise in red cells, whereas later, when erythroblastosis was marked, the injections failed completely. In this case transfusions were followed by greatly increased urobilin excretion.

In the case of J. C. the injection given on 3/2/34 had no apparent effect. This was probably because the maximum effect had already been attained and another injection caused no further fall in urobilin, but only a continuation of the rise in red cells and hemoglobin.

Finally, failure may be due to the lack of potency of the material used for injection. This factor in failure is entirely unstudied up to the present, but the fact that the more purified product is less effective than the less purified makes it probable that something in the preparation influences the potency of the final product.

In view of the possible factors that limit the effect of transfusions or plasma concentrate injections, we have a right to consider the positive results as of greater significance than negative results, provided the number and nature are such as to exclude coincidence.

Since this seems to be the case, negative results here recorded are facts to be explained, not objections which have to be met.

The principal measurable effect is on total urobilin excretion. From the work reported in the first paper showing that the urobilin excretion in the conditions represented here is a measure of blood destruction, it may be concluded that transfusions and injections of plasma concentrate are factors in the reduction of the amount of blood destruction. In the majority of cases the reduction in blood destruction was accompanied by a rise in red cells, but in some the red cells failed to rise, nor was the extent of the rise in red cells always parallel to the fall in urobilin excretion. This indicates the presence of other factors in these cases besides blood destruction. What these other factors are has not been ascertained, but they appear to be related to erythropoietic activity and especially to the presence of erythroblastosis.

The variability in red cell response limits the therapeutic applications of the injections. In fact, the principal interest of these results lies in their bearing on ideas concerning the etiology of sickle cell anemia and congenital hemolytic anemia; for they indicate the possibility that in these conditions something is lacking that is present in normal blood plasma. The first thought is that it might be some substance having antihemolytic action, but such a conclusion would not be justified at present. We know too little yet concerning the mechanism of hemolysis in these cases, especially the part played by faulty erythropoiesis or the influence of the spleen, to specify the point at which this unknown substance acts. It brings us face to face with the possible hormonal or humoral regulation of blood formation and destruction and therefore with the possibility that hemolytic anemia of constitutional origin may be in part dependent on a disturbance in some internal secretion. Further speculation regarding the nature of this "antihemolytic" substance is unprofitable at the present time.

SUMMARY

Starting with the observation that in sickle cell anaemia a transfusion was followed by a marked temporary reduction in the rate of blood destruction, it has been found that there is in normal plasma a substance that appears to have a specific influence in reducing the rate

of blood destruction not only in sickle cell anaemia but probably in congenital haemolytic jaundice as well. The effective substance has thus far been found in the ether-insoluble fraction of concentrated protein-free plasma and is effective in minute amounts. It is too soon yet to say whether this material will have any therapeutic application; thus far the therapeutic results have been disappointing. In explanation of the results it is suggested that in sickle cell anaemia and possibly also in congenital haemolytic jaundice there may be a lack of some substance normally present, the function of which is to aid in maintaining the normal equilibrium between formation and destruction of blood.

STUDIES OF HAEMOLYTIC ANAEMIA

III. Pig's Plasma as a Source of Anti-haemolytic Factor

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In the paper preceding this one in this series of studies of haemolytic anaemia, it was shown that the urobilin excretion could be temporarily reduced in amount by transfusion, plasma intravenously, concentrated protein-free plasma, and finally concentrated protein-free, fat-free plasma. All the preparations used up to this time were obtained from normal human blood. Several immediate problems were, of course, raised by this finding, but before anything further could be done it seemed advisable to have a source of plasma more abundant than could be obtained from colleagues, or cheaper than that of professional donors. It was therefore decided to use animal plasma, and the pig was selected merely because of a convenient source near at hand.

The method of preparation is as outlined before. Essentially it consists in precipitating the proteins of the plasma with 95% alcohol; evaporating the alcoholic solution to dryness in a vacuum, in order to avoid raising the temperature unduly until it was learned what effect heat would have on the activity; extracting the residue with alcohol; concentrating this solution and filtering off the last of the proteins; evaporating to dryness and extracting the lipoids with ether. The residue after the ether extraction was taken up in alcohol and stored in the refrigerator. When an injection was to be made, a portion of the alcoholic solution corresponding to a known amount of the original blood was evaporated and taken up with a small amount (1 cc. or less) of freshly distilled water for intramuscular injection.

In order to save space Tables have been omitted and the results have been recorded in Charts. In recording results it may be noted that

* Holder of a Latin-American Fellowship of the Association of University Women.

the daily urobilin excretion is reported as mgs. per gm. of haemoglobin. As explained previously, this appears to give a better idea of the severity of the haemolytic process, and allows comparison between patients of different ages. In evaluating the severity of the haemolytic process it should be explained that normally the urobilin excre-

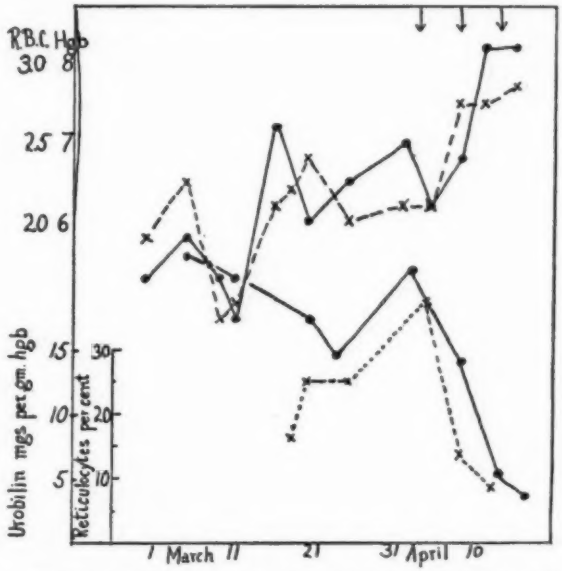

CHART 1. COURSE AND EFFECT OF TREATMENT IN CASE OF T. H. (CASE 1)

Explanation of symbols: The solid line in the upper part represents haemoglobin, the broken line in the upper part represents red cells, the solid line in the lower part represents urobilin excretion, the broken line in the lower part represents reticulocytes, the arrows at the top of the chart denote injections of plasma extract.

Note: From March 9th–11th there was a slight increase in numbers of erythroblasts, which, however, never reached more than 1,000 per cu.m.m.

tion lies between 0.2 and 0.4 mg. per gm. of haemoglobin, but that a fifty or even a hundred per cent rise has little significance. Rises to 1 or 2 mgs. occur frequently in various infections. One may consider 5 mgs. as indicating a moderate degree of haemolysis for sickle cell anaemia or congenital haemolytic anaemia. 10–15 mgs. indicate a

relatively severe process. Excretions of over 20 mgs. mean extremely severe haemolysis occurring only in the haemolytic crises. I have personally never seen an excretion above 30 mgs. per gm. of circulating haemoglobin.

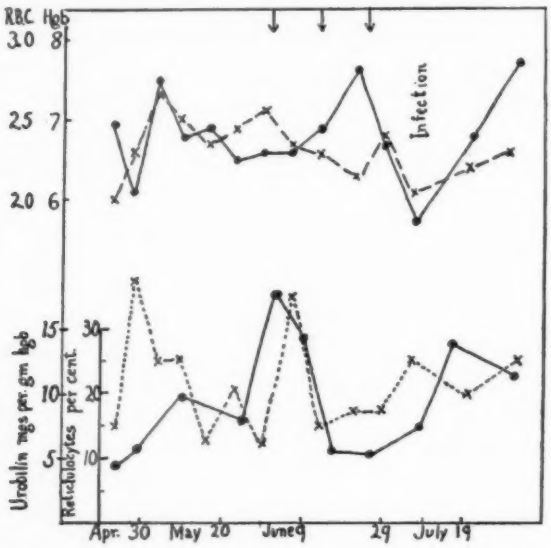

CHART 2. COURSE AND EFFECT OF TREATMENT ON CASE OF E. C. (CASE 2)

Note: This child was subject to frequently repeated crises of considerable severity. Since the majority lasted longer when untreated than the one recorded here, it is thought that the injections could be considered to have had an effect in shortening this crisis.

The investigation was carried out on four cases of sickle cell anaemia, brief summaries of which follow:

Case 1. T. H. Col. Aged 3 years. Sickle cell anaemia. The onset of the active phase of the disease was about three months before admission. It was rather gradual, with enlargement of the abdomen and loss of weight rather than by a severe crisis. He was brought to the hospital because of fever and diarrhea and on admission was found to have a fairly severe sickle cell anaemia. During his stay he improved markedly, especially after the plasma concentrate injections, then suddenly, without warning, developed an acute crisis and died in a few hours,

before a transfusion could be given. At autopsy it was thought possible that he had bled to death into his spleen. There was no haemoglobinaemia or haemoglobinuria, and it was felt that these would almost surely have occurred had destruction of blood cells been sufficient to account for the rapidity of the fall in haemoglobin (8.2 to 2.6 g. in 8 hours).

Case 2. E. C. (This case was included in the previous paper, so that the history need not be repeated.)

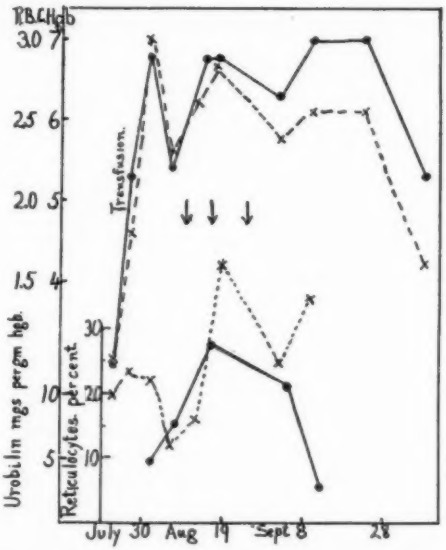

CHART 3. COURSE AND EFFECT OF TREATMENT IN A. P. (CASE 3)

Note: This was the youngest patient studied thus far. There was marked tendency to erythroblastosis, and frequent severe crises. Because of the fact that this patient's urobilin excretion was relatively high when untreated, it is felt that the reduction following the injections is significant.

Case 3. A. P. Col. Aged 5 mos. Sickle cell anaemia. The active stage of the disease was discovered at an unusually early age (4½ mos.). The characteristics were those of sickle cell aneamia occurring in infancy—a tendency to severe crises fairly frequently repeated and marked erythroblastosis. Aside from the severe anaemia almost constantly present, the baby did remarkably well, gaining weight like a normal child.

Case 4. R. W. (Included in the previous paper.) The child was admitted

for the study reported here and was not sick until March, 1937, when she developed a number of infections, which so interfered with the study that it was abandoned. Her sickle cell anaemia was mild and she had no tendency to develop haemolytic crises.

The clearest results are those obtained in the case of T. H. (Chart 1) on one occasion, and in R. W. (Chart 4) on two occasions, in which the effects on urobilin excretion and on the red cells are definite. In the case of T. H. the only doubt arises from the fact that just before

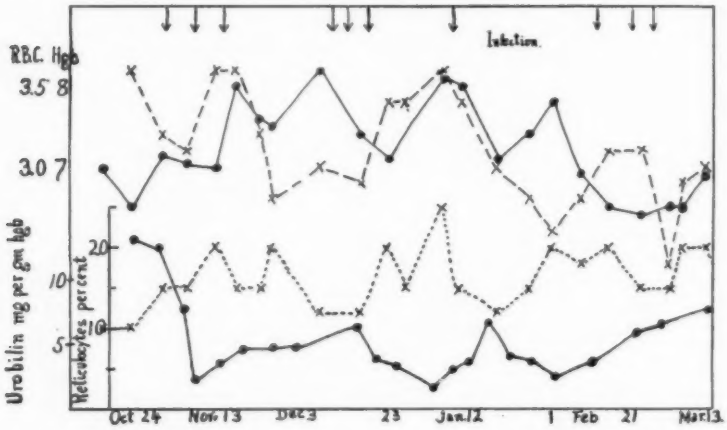

CHART 4. COURSE AND EFFECT OF TREATMENT IN R. W. (CASE 4)

Note: Treatment in this case was definitely successful up to January. The injection of January 5th had a very doubtful effect. All the injections up to this one were made with material from blood obtained in October. The last recorded injections in February were made with fresh material and were entirely unsuccessful.

the injection there had occurred a temporary but well marked fall in urobilin excretion and rise in red cells unrelated to any therapeutic procedure. This spontaneous remission appears to have marked the end of the crisis for which he was admitted, a crisis characterized not only by a high urobilin excretion but also by a considerable degree of erythroblastosis. There is, therefore, a remote possibility that

remission following the injection of the plasma concentrate was a coincidence.

In the case of R. W. the repetition of the effect renders coincidence out of the question, and as was stated in the previous paper, the failure of a positive result should be considered rather a fact to be explained than something which renders doubtful the success of other injections. The injection of January 5th was followed by a fresh fall in urobilin excretion, but the presence of an infection may easily have caused the fall in red cells. On the other hand, the material used was the same as that made up in October, and it is possible that during this time it might have deteriorated. The injection made in February was made with material from a fresh lot of blood and failed completely, both in this case and in that of A. P. Whether for some reason this fresh lot of blood was lacking in the anti-haemolytic factor, or whether it was the infections present in both these children during the winter months that interfered, it is not possible to say. It was noted that when the plasma from which the material was prepared was turbid (probably from lipemia), the injections failed to produce any effect. This possibility will have to be studied further.

In the case of E. C. (Chart 2), the apparent effect could possibly have been a coincidence, since the rise in urobilin might have been merely a short-lived crises. Crises of such short duration are not common, but they sometimes occur.

In the case of A. P. (Chart 3) the injections were followed by a fall in urobilin excretion. The study could not be completed, owing to the removal of the child from the hospital, but the subsequent fall in red cells and haemoglobin indicate the probability of a later rise in urobilin excretion. As has been indicated, a later study of this case gave negative results, but it is not known whether the failure was due to the presence of infection and erythroblastosis in the child or to the lack of potency in the plasma concentrate.

These results as reported here confirm those that have been reported in the previous paper, and advance the investigation one more step. We had hoped to be able to test the effect of heat on the potency of the concentrate, but the lack of cases and the fact that the patients we were investigating had to be discharged from the hospital before

the end of the study, prevented this. The irregularity of the effect on red cells and haemoglobin as well as the interference of infections, limit the therapeutic value, although the fact that in some cases a good therapeutic result was obtained keeps alive the possibility that some day we may be able to use these plasma concentrates more effectively.

At present the principal result is to make possible investigations which may lead to a better understanding of the factors responsible for haemolytic anaemia.

THE INHIBITORY EFFECT OF SULFANILAMIDE ON THE DEVELOPMENT OF EXPERIMENTAL TUBERCULOSIS IN THE GUINEA PIG

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Submitted for publication December 1, 1937

In view of the now familiar beneficial effect of sulfanilamide (para-amino-benzene sulfonamide) in several types of bacterial infection, it seemed advisable to test the efficacy of this drug upon infection with the tubercle bacillus. The present report deals with part of a study directed toward this end.

59 guinea pigs, averaging about 400 gms. in weight, were used in these first experiments, the object of which was to determine whether the proliferation of the tubercle bacillus in the animal body could be inhibited by the drug. All animals were kept under the same conditions of nutrition and laboratory care. Each animal was infected subcutaneously in the groin with 0.1 cc. of a suspension of virulent human type tubercle bacilli containing an average of 5 bacilli per oil immersion field. 31 of the infected animals were treated by the oral administration of crystalline sulfanilamide ("Prontylin Powder," Winthrop Chemical Co.) and 28 were kept untreated as controls. The treated animals received the drug daily from the beginning to the end of the experiment. Measured doses were deposited in the cheek pouch; the animals kept under observation retained the powder thus administered. Whenever a treated animal died a control was killed for comparison of the extent of the lesions. All survivors were killed at the end of 5 weeks in Series 1, and at the end of 6 weeks in Series 2 and 3. These three series represent three separate experiments carried out consecutively.

Series 1 comprised 17 infected animals, 10 of which were given 100 mgs. of sulfanilamide in a single dose each day, while 7 were kept untreated as controls.

Series 2 consisted of 12 infected animals, 6 of which received 200 mgs. of the drug daily in two doses, 100 mgs. in the morning and 100 mgs. in the evening. 6 animals were kept untreated as controls.

Series 3 consisted of 30 infected animals, 15 of which received 500 mgs. of the drug daily in four doses, as follows; 100 mgs. at 9 a.m., 100 mgs. at 2 p.m., 100 mgs. at 6 p.m., and 200 mgs. at 11 p.m. The remaining 15 animals were kept untreated as controls. In this series the administration of the drug was begun

three days before the bacilli were injected. Later, because of the appearance of toxic symptoms, the dose was reduced to 300 mgs. per day during 3 days in the first part of the 6th week. Two treated animals died in the first four days after infection and were discarded.

The results of these experiments may be briefly summarized as follows:

In Series 1, no appreciable difference could be discerned macroscopically or microscopically between the treated animals and the controls. 100 mgs. of the drug administered in a single dose once a day was ineffective against the numbers and virulence of the bacilli used.

In Series 2 there was a definite difference between the treated animals and the controls. In the former, the amount of macroscopic and microscopic tuberculosis throughout the body was significantly less than in the latter. All of the controls had macroscopic tuberculous foci throughout their enlarged spleens, whereas in the treated animals macroscopic lesions were seen in the spleen in only one case. In fig. 1 are shown the spleens of animals of this series. Tubercles were found microscopically in the tissues of all the treated animals, but their numbers and extent were markedly less in the treated group than in the control group. 200 mgs. of the drug daily, divided into two doses, had a definitely inhibitory effect upon the infection.

In Series 3 the differences between the treated and the untreated animals were much more striking (fig. 2). After 3 weeks it was evident on palpation that the sites of infection and the regional lymph nodes of the untreated animals were much larger than those of the treated animals, and the difference became progressively more striking with the passing of time. Indeed, in many of the treated animals no definite palpable local lesion or enlargement of the regional nodes could be discerned, whereas all of the untreated animals had large local lesions and regional nodes. At autopsy in the fifth and sixth weeks the controls showed large local lesions and regional nodes, greatly enlarged tuberculous tracheobronchial nodes and spleens, whereas in the treated animals the local lesions and regional nodes were minute in comparison. Treated animals coming to autopsy in the fifth week showed no splenic enlargement whatever, and remarkably minute tracheobronchial nodes which were even smaller than those of normal animals of the same weight. In none of the viscera

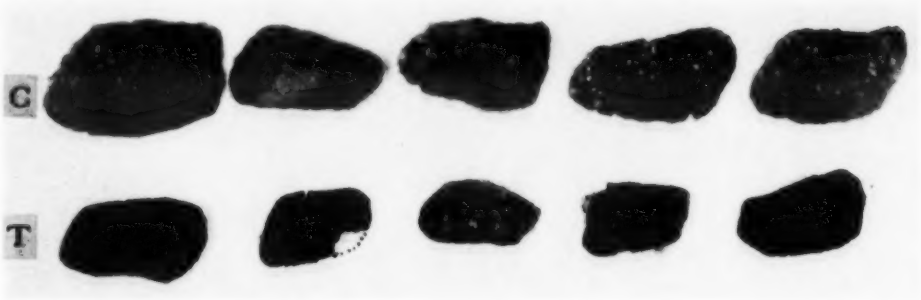

FIG. 1. SPLEENS OF ANIMALS OF SERIES 2 AT END OF 6 WEEKS AFTER INFECTION

The untreated controls are shown in the upper row C; the treated animals in lower row T. One treated and one control animal not included in the photographs showed at 5 weeks an entirely comparable difference.

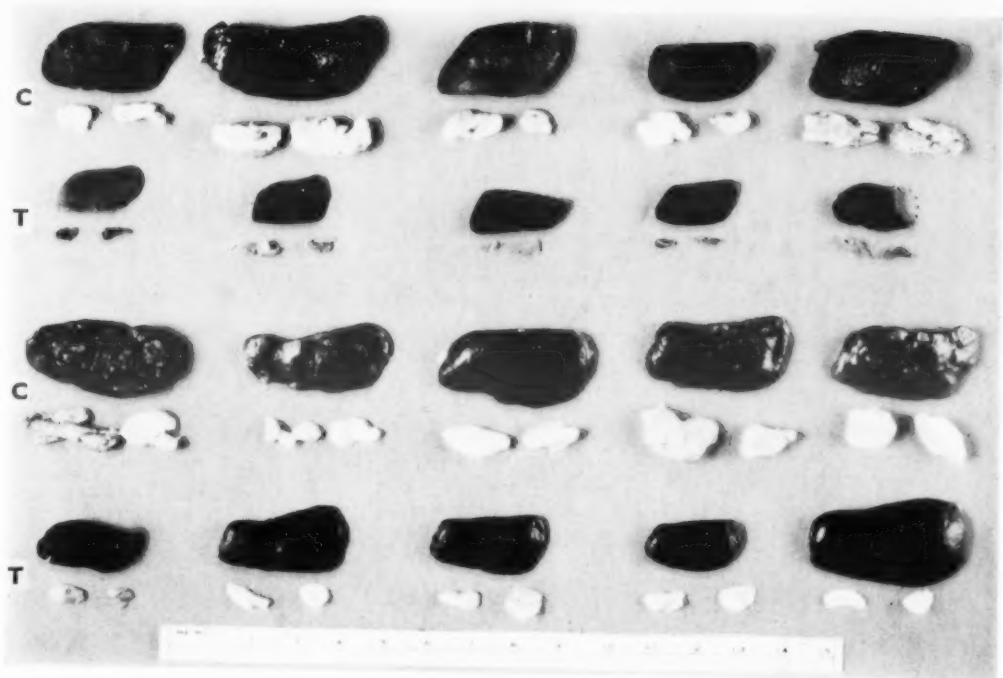

FIG. 2. SPLEENS AND TRACHEOBRONCHIAL LYMPH NODES (THE LATTER CAREFULLY DISSECTED FREE FROM SURROUNDING TISSUES UNDER BINOCULAR MAGNIFICATION) OF ANIMALS OF SERIES 3

The untreated animals are shown in rows C, the treated in rows T. The first four sets of organs of treated and corresponding control animals in the upper rows are from animals coming to autopsy in the 5th week of the infection; the remainder are organs from animals coming to autopsy at the end of the 6th week. As stated in the text, minute tubercles were found microscopically in the spleens of all of the animals dying in the 6th week, and in 3 of 4 of those dying in the 5th week. (Photographs by Mr. Milton Kougl)

of the treated animals were macroscopic tuberculous lesions seen. Of the 6 animals in the treated group coming to autopsy in the sixth week only one showed macroscopic tuberculous foci in the spleen, but neither the number nor the size of the foci approached that found in the untreated animals at this period (fig. 2). No macroscopic foci were found in the spleens, lungs or livers of any of the other treated animals. In only one treated animal was the spleen as large as that of the controls, and in this case no macroscopic tuberculous foci were visible on the surface or on section, and no foci were visible in the lungs or liver. Microscopically, this enlarged spleen showed scattered epithelioid cell tubercles with no caseation. Similar microscopic tubercles were found in all treated animals at the end of the 6th week and in 3 of 4 animals in the 5th week, but their average numbers and extent were markedly less than those in the untreated group. In the sections of the treated group caseation was conspicuously absent, in contrast to the prominent caseation in the more extensive lesions in the untreated animals. In only 3 treated animals was any caseation found microscopically, and here it was of restricted degree, *i.e.*, a minute necrotic focus in one small tubercle in the spleen of an animal that died at the end of the 5th week, a similar focus of caseation in one tubercle in the spleen of one animal, and two foci in tubercles in a tracheobronchial node of another animal killed at the end of the 6th week. The section of the spleen of this latter animal showed only two minute epithelioid cell tubercles without caseation. Large areas of caseation were the rule in both the spleens and tracheobronchial nodes of the controls coming to autopsy at 6 weeks.

Stainable bacilli were present in the lesions of all treated animals, but there were many more in the tissues of the untreated controls. Tissue emulsions from representative animals have been injected into normal guinea pigs to test the viability of the bacilli in the organs of the treated animals.

It is clear that in none of the treated animals did the drug prevent completely the growth of the bacilli. Whether the less extensive lesions and fewer bacilli in the treated animals are the result of inhibition of rate of growth or of more effective destruction of the bacilli we are as yet unable to say. It may be recalled that while the sulfanilamide is remarkably effective against haemolytic streptococci in

week, and in 3 of 4 of those dying in the 5th week. (Photographs by Mr. Milton Kougl)

man, Long and Bliss (2) found that in the mouse the streptococci multiply for a time after therapy has been instituted, and that the drug is often ineffective in producing complete sterilization in that highly susceptible animal. The majority of 48 mice treated with 6 to 8 times the human therapeutic dose for as long as two weeks after infection, and apparently cured, succumbed some weeks later to streptococci left alive in their tissues. In the natively more resistant human being permanent cure in streptococcal infection is more readily achieved by the drug. The great susceptibility of the guinea pig to the tubercle bacillus is well known.

Since we could find no studies on the toxicity of sulfanilamide for guinea pigs, we were forced to seek the optimal dosage empirically, and in Series 3 we undoubtedly gave too large a dosage, for 9 of the treated animals became ill, developed symptoms noted in other species as a result of over-dosage (1) and died. Three of these deaths occurred in the third week of the infection, four in the fifth week and two in the sixth week. In Series 2, in which a definite but less marked effect upon the infection was produced, the dose was apparently below the toxic level. The fact that in the animals that died from the effects of the drug the toxic symptoms did not appear until several weeks after treatment was begun indicates that in the guinea pig the drug has a cumulative effect, and it is possible that after an effective blood level has been attained the dosage could be reduced with no loss of therapeutic effect and with less danger of toxic effects.

The dosage used in Series 3, in which the most marked inhibitory effect on the infection was obtained, was much larger in proportion to the body weight than that recommended by Long and Bliss (2) in the treatment of streptococcal and meningococcal infections in the human being. The limit of tolerance of the human being for the drug is not yet known. Southworth (3) has administered 0.11 gm. per kilogram daily for a month in the human being. The animals of our Series 3 received about ten times that dosage, and those of Series 2 about four times that dosage. It should be recalled that in the experimental work on the beneficial effect of the drug on streptococcal and meningococcal infections the successful treatment of the animals required up to ten or more times the dosage that proved to be effective in the human being (4) (5) (6) (8).

In relation to the dosage necessary in guinea pigs, it may be stated that Dr. E. K. Marshall, Jr. has kindly determined the concentration of sulfanilamide in the blood of some of our animals at various intervals after administration of the drug, by the method previously developed by him and his associates (7), and he has found that the guinea pig acetylates a much larger proportion of the drug than does the human being. This is important in relation to the dosage required for effective results, because the acetylated derivative has been found to be almost inactive against other bacteria (7), though it is even more toxic than the active free sulfanilamide (9). The guinea pig, therefore, requires a much larger proportional dose of sulfanilamide than does the human being in order to attain and to maintain the same blood level of the free, active drug; and because of its greater power to acetylate the drug this animal is subjected to a greater hazard as regards toxic effects. We wish to emphasize strongly, however, that no conclusions regarding the possible effects of sulfanilamide in human tuberculosis are warranted from the experiments here reported. Whether or not the drug will have any similar inhibitory effect upon tuberculosis in the human being cannot be surmised without pointed investigation under carefully controlled conditions with the most painstaking efforts directed toward the avoidance of the toxic effects that are now recognized as hazards in the use of this drug in the human being (10) (11).

It is obviously desirable to determine the minimum dosage and period of treatment necessary to produce the maximum inhibitory effect upon this infection; and also to determine the result of delaying treatment in the experimental animal until tuberculous lesions are well established, and the effect of withdrawing the drug after various periods of treatment. We shall report later on studies in progress dealing with these points.

SUMMARY

Under the conditions of the experiments here described, sulfanilamide has exerted a striking inhibitory effect upon the development of tuberculosis in the highly susceptible guinea pig.

It is emphasized that no conclusions should be drawn regarding any possible effect of this drug upon human tuberculosis until the latter

problem has been subjected to a carefully controlled study, carried out with due regard for the toxic potentialities of the drug.

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A PRELIMINARY REPORT ON A PLATELET-REDUCING SUBSTANCE IN THE SPLEEN OF THROMBOCYTOPENIC PURPURA

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(Submitted for publication December 3, 1937)

The purpose of this brief report is to call attention to a substance which was found in the spleen of three cases of thrombocytopenic purpura. This substance had the property of being able to reduce the platelet count in the circulating blood of a normal rabbit as much as 90 per cent. In view of the relative infrequency with which cases of thrombocytopenic purpura occur, it would seem justifiable to make this preliminary statement in order that other investigators may check our findings and contribute to a further study of this substance. A full report will be offered later. The spleens were obtained through the coöperation of Dr. Dean Lewis, whose idea it was that extracts might be made from such clinical material and that the disease may thus possibly be reproduced experimentally.

The method for making the extract which contained the thrombocytopenic substance was very simple. The spleen was taken directly from the operating room and ground up in a food chopper. To this ground-up material in a rubber sealed jar was added twice its volume of reagent acetone. The jar was shaken occasionally for several days and after about two weeks the supernatant fluid was filtered away and the acetone distilled off, leaving a small amount of a thick brownish material which was deposited on the walls of the distilling flask. One hundred cubic centimeters of distilled water, added to the flask, were shaken vigorously so that as much as possible of the brown substance was taken up by the water. This solution was filtered. The filtrate was of a yellowish light brown color and cloudy, but it did not show a precipitate after standing in the ice box for weeks.

The test animal was the rabbit, a new animal being used for each experiment. Of the first preparation 20 c.c. were injected into the ear vein and at the end of 24 hours the platelet count, as determined by the direct method, using the fluid of Rees and Ecker, was reduced from the pre-injection level of 620,000 platelets per cubic millimeter of blood to 58,000. At the end of 36 hours the platelet count was back to normal again. The bleeding time was greatly prolonged at the height of the reaction. No purpuric spots in the skin were noticed.

The extract of the second thrombocytopenic spleen gave a platelet fall from 640,000 to 70,000 in 24 hours, and the extract from the third thrombocytopenic spleen gave a fall from 610,000 to 100,000 in 24 hours.

It was soon found that the original injection of 20 c.c. was needlessly large and that 5 c.c. or even 3 c.c. would produce almost as much thrombocytopenia as the much larger amount.

Repeated injections of the extract caused the platelet count to remain at about 70,000 for a period of 9 days. Four injections were given during this period and it is probable that this procedure would have maintained this low count for a much longer period.

Four control extracts were used and were prepared by a method identical with that used for the thrombocytopenic spleens. The first control extract was prepared from thyroid tissue; the second from a myomatous uterus; the third control was prepared from a spleen showing Banti's disease; and the fourth control was from a spleen of a patient suffering from hemolytic jaundice. All of these four control extracts were made from tissue removed at operation, and virtually no decrease in the number of platelets was noticed following the injection of these respective extracts. These control extracts show that the spleen in thrombocytopenic purpura contains a substance which causes a very marked thrombocytopenia. Incidentally, the red and white cell counts were not changed whereas the platelets were reduced in number, thus indicating a specificity for the thrombocytopenic substance. It is suggested that the term "thrombocytopen" be given to the thrombocytopenic substance described above.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Health Education of the Public. By W. W. BAUER and THOMAS G. HULL. 227 pp. Illus. $2.50. (*W. B. Saunders Company, Philadelphia, 1937.*)

This should prove to be a very valuable handbook for use by those who are interested in the health education of the adult. Out of their experience the authors give the health educator details of "how" to educate the public rather than "what" to teach them, and throughout they deal solely with the various methods by which the educator may reach his audience. Separate chapters are devoted to various means available, such as radio, newspapers, meetings, motion pictures, etc., and each is considered as to its limitations, advantages, and disadvantages. The technique which in the light of experience has been found most satisfactory is detailed for each approach to the public. Where necessary, the book has been illustrated to bring out the various points in the technique: this is particularly true in the chapter dealing with stereopticon slides. An unusual and quite valuable feature of the book is the information as to where the educator may obtain the materials, such as books, periodicals, motion pictures, and the like, necessary to carry on any educational program. While each technique is dealt with separately, the authors have a chapter on the importance of correlating all parts of the program. Admitting that the results of any educational program of adults is difficult to appraise, the authors suggest criteria for measuring health progress in a community. This chapter could well have been omitted without loss to the value of the book.

R. L. G.

Contributions to the Microscopic Anatomy of the Pancreas. By PAUL LANGERHANS [Berlin, 1869]. Reprint of the German original with an English translation, and an Introductory Essay by H. MORRISON, M.D. 39 pp. $1.00. (*The Johns Hopkins Press, Baltimore, 1937.*)

It is of historical importance to have this reprint of the rather inaccessible inaugural dissertation of 1869, in which Langerhans described his very careful study of the histological characteristics of the pancreas, recognizing for the first time the existence of small masses of cells different from those of the acini of the gland which are connected with the ducts. Great honor is due to Laguesse who realized that they were first described by Langerhans and named them the islands of Langerhans, since, except when an eponym is conferred, fame is so often transitory or even transferable.

Since the paper of Laguesse, in 1893, the advance in knowledge of the function of these cells through the work of Ssobolew, Schulze and Opie, and later of Banting, Best, McLeod, and Collip has thrown such light on the activity of insulin which they produce, that the problem of diabetes mellitus has been regarded as practically settled, but more recent studies have shown that there are complex relations with the other endocrine organs which leave it still obscure in many though not in all cases.

Dr. Morrison's biographical note with its photographic illustrations of the Langerhans family adds greatly to the interest of the publication.

W. G. M.

An Introduction to Dermatology. By RICHARD L. SUTTON and RICHARD L. SUTTON, JR., 3rd ed. 666 pp. Illus. $5.00. (*C. V. Mosby Co., St. Louis, 1937.*)

This is the third edition of a relatively small volume which is intended primarily for students. Its enlargement to include several additional diseases is, to the reviewer, no particular improvement. A book for students should be limited to essentials and to descriptions of such conditions as are likely to be seen during the course of instruction or time of practice. Such affections as onion mite dermatitis, grasshopper dermatitis and others included in the new edition might better have been left for books of encyclopaedic proportion.

Unnecessary space is devoted to too many methods of therapy which have not proved of value, and to unsupported theories of etiology. Such additions merely serve to confuse the student and to divert his attention from the essentials. One glaring mistake was noted on page 600 where the *acne bacillus* was stated to be "probably the Pityrosporum Ovale".

The book, however, in general, follows along the usual lines of dermatological texts and as a whole can be recommended. The photographs are very good and the student or general practitioner will find them useful.

L. W. K.

The Larynx and its Diseases. By CHEVALIER JACKSON and CHEVALIER L. JACKSON. 555 pp. Illus. $8.00. (*W. B. Saunders Company, Philadelphia and London, 1937.*)

This volume which covers the clinical experience of the authors from the "horse and buggy" days to the present time is a valuable addition to medical literature.

In thirty-one chapters every conceivable condition occurring in the larynx and the surrounding tissues is covered. The text contains many illustrations and photographs bringing out important points, with eleven plates of paintings by the authors, and accompanying them are interesting histories of the cases illustrated. Many of the methods described for treatment and operative procedures were developed originally by the authors and their associates.

To laryngologists the chapter on the treatment of laryngeal stenosis is especially

interesting. On the other hand, the one on operations for malignant diseases of the larynx is comparatively short, and there is a feeling of surprise that a separate chapter has not been devoted exclusively to foreign bodies of the larynx.

The type is clear and the style typically Jacksonian. The book is to be recommended highly to the general practitioner and to the laryngologist as a working manual and for reference.

E. N. B.

Inhalation Anesthesia. By ARTHUR E. GUEDEL, M.D. 172 pp. $2.50. (*The Macmillan Company, New York, 1937* (*Macmillan Surgical Monographs*).)

The principles of inhalation anesthesia and the occurrence of anesthetic accidents are stressed from the standpoint of clinical practice. Intentionally the more complicated physiological and pharmacological mechanisms are not exhaustively discussed. However, these factors are adequately described in this concise and practical handbook. Certainly some of the deaths caused by anesthesia may be avoided if this book is read by prospective anesthetists.

There is a brief discussion of the mechanism of inhalation anesthesia as governed by the laws of diffusion of the different gases and vapors used. This is followed by a description of the four stages of anesthesia. The third stage, that of sufficient depth for surgical procedures, is subdivided into three planes. Smooth muscle loses its tone in the lower third plane. At this level of anesthesia intestinal peristalsis ceases and the arterial walls relax so that shock comes on rapidly. Fifteen minutes of anesthesia in the third plane will produce greater circulatory depression than two hours of anesthesia in the first plane. The depth of anesthesia required for various operative procedures, and the selection of anesthetic agents are discussed.

The second part of the book deals with anesthetic accidents: cardio-vascular accidents, respiratory failure, and actual mechanical obstruction of the air passages. The individual case descriptions assist in rendering the text very instructive. Artificial respiration, although it should be instituted as soon as respiration ceases from any cause, is frequently fatally delayed.

Finally, there is a chapter on anesthetic explosions with advice as to dangerous mixtures and suggested practical measures to prevent ignition by static spark.

I. R. T.

Tweedy's Practical Obstetrics. Revised by BETHEL SOLOMONS and NINIAN MCI. FALKINER. Seventh Edition. 773 pp. Illus. $8.75. (*Oxford Medical Publications, Oxford University Press, London, 1937.*)

This edition is the seventh of Tweedy's "Practical Obstetrics", the first of which appeared in 1907. It has been materially revised and brought up to date by Solomons and Falkiner. The revision has been accomplished without destroying the sound background and essential conservatism which has always marked the book. For readers in this country it is of interest as an example of the best

of obstetrics as practised in the British Isles and particularly at the Rotunda Hospital. Furthermore, it affords a contrast between midwifery methods on the two sides of the Atlantic. As a reference work it deserves a place in all obstetrical libraries.

C. H. P.

For Stutterers. By SMILEY BLANTON and MARGARET GRAY BLANTON. 191 pp. $2.00 (*D. Appleton-Century Co., New York, 1936.*)

A book intended primarily for stutterers themselves but interesting also to everyone concerned with speech defects from any angle. Seven chapters are devoted to a discussion of the physiological and psychological mechanisms underlying speech and its aberrations. The parts of the body utilized in speech have their most vital use in other fields of activity, such as swallowing and breathing; and in emergencies, speech must surrender them in favor of these more primary functions. Faulty speech is a manifestation of an underlying nervous condition which may have innumerable causes. Fear and anxiety block the rhythm of speech. This becomes fixed by habit and by a further conditioning of the original block. Therefore, the Blantons believe, it is futile to give any treatment that does not have as its aim the elimination of the underlying emotional difficulty. A thorough study must be made of the physical and personality make-up, of the environment, and of the psychological blocking that is at the root of the difficulty. This may be accomplished by psychoanalysis, by individual guidance, or, in organized groups, by directed activities, including a daily period of personality analysis. The rôles played by the parents, the teacher, the public and the stutterer himself are discussed because, at basis, relief for the stutterer is to be considered a community project.

Other theories of treatment are reviewed and evaluated. Those which treat stuttering primarily as a simple speech defect are taken to task. Phonetics, drills and direct suggestion do not relieve the underlying emotional difficulty and are contra-indicated because they focus attention on the speech itself. Likewise, retraining the hand, on the assumption that the difficulty is due to changing from lefthandedness, should be subordinated to the establishment of emotional adjustment. Relaxation is effective because it reduces tension and permits a reconditioning of speech in a relaxed state. But fundamentally the problem is one of personal adjustment and it is on this point of attack that any real therapy must be grounded.

E. S. C.